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EDITORIAL

XV Annual Scientific Meeting of the Butantan Institute
Internationalization of Brazilian Science: Challenges and Paths to the Butantan Institute

On behalf of the Organizing Committee we welcome all participants of the XV Annual Scientific Meeting of the Butantan Institute, whose theme is **"Internationalization of Brazilian Science: Challenges and Paths to the Butantan Institute".**

The Scientific Meeting is an event that has allowed, annually,the integration among researchers, professors, students, staff and other professionals of this Institute. It has been a unique opportunity to exchange experiences, to discuss new ideas, to establish new collaborations and to socialize, in a friendly environment.

The undeniable growth of Brazilian science and its international projection have been constantly discussed. This meeting brings us the reflection on the current and future presence of our institution and its image abroad, as well as the opportunity to discuss ways to expand the horizons of our science, research and production across national borders.

The goal is to gather international relations experts in research, in production and promotion agencies, broadening the discussion in these areas and the possibilities of international partnerships.

The scientific program will be divided into three thematic sessions, dedicated to discuss the Institute challenges for its internationalization. There will also be three panel sessions and Young Scientists Awards (Scientific Initiation and PAP Program, Master and Doctoral Degrees). This year, as a novelty in this event, the Innovation Award, which will award studies with innovative character of the research and/or its results or practical applicability, considering the Brazilian legal requirements for patentability, will be presented.

In addition to this program schedule, the Satellite Event of the Graduate Program in Toxinology will occur on December 03, whose theme is “Animal Venoms and Toxins: Interaction with molecular targets”.

We are awaiting your presence, wishing you to have a great event.

Dr. Gisele Picolo
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Director of the Scientific Division

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XV Annual Scientific Meeting of Instituto Butantan December4th to 6th, 2013

Internationalizationof Brazilian Science: Challenges and Pathsto the Butantan Institute

SCIENTIFIC PROGRAM

Tuesday 12/03/2013

IV Satellite Symposium of the Post-graduation Course in Toxinology – Butantan Institute "Animal Venoms and Toxins: Interaction with molecular targets"

Coordinators: Dra. Dra. Ana Marisa C. Tavassi and Dra. Catarina F.P. Teixeira

14:00 - 14:50 **Lecture**
"Snake venom disintegrins as tools for comprehension of cell adhesion and migration"
Dra. Heloisa Selistre (UFSCAR)

15:00 - 15:25 **Short Talk**
"Comparative studies on snake venoms from *Bothrops* complex: Phylogeny and reactivity with antivenom"
Leijiane Figueira de Sousa
Graduate Student, PPGTox - Instituto Butantan

15:30 - 15:45 **Coffee break**

15:45 - 16:10 **Short Talk**
"Molecular mechanisms involved in biosynthesis of prostaglandin induced by a metalloproteinase from *Bothrops* snake venom in synovial fibroblasts"
Mariana do Nascimento Viana
Graduate Student, PPGTox - Instituto Butantan

16:15 - 16:40 **Short Talk**
"Role of dinein in anti tumor mechanisms triggered by Amblyomin-X in melanoma cells and pancreatic adhenocarcinoma"
Mario Thiego Fernandes Pacheco
Graduate Student, PPGTox - Instituto Butantan

Wednesday 12/04/2013

XVAnnual Scientific Meeting of Instituto Butantan – Internationalization of Brazilian Science: Challenges and Paths to the Butantan Institute

09:00 - 09:30 **Opening Session**

09:30 - 12:30 **Thematic Session I: Research in the Global Scenario**

Coordinator: Dra. Yara Cury (Instituto Butantan)

09:30 – 09:40 Research at Instituto Butantan:state of art

09:40 - 10:20 **"A Cooperação Sul Sul e a Internacionalização do Desenvolvimento Tecnológico e Inovativo"**
Dr. Márcio Porto – Embrapa

10:20 - 11:00 **"Possibilidades de internacionalização: Experiência da USP"**
Dr. Aluísio Augusto Cotrim Segurado – Universidade de São Paulo

10:50 - 11:20 **Coffee break**

11:20 – 12:00 **A short disquisition on how to internationalise a research institution**
Dr. Antonio Coutinho - Calouste Gulbenkian Foundation

12:00 – 13:00 **Lunch**

13:00 – 15:00 **Poster Session I**
1. Venoms and Envenomations
2. Biochemistry
3. Pharmacology
11. PIBIC/PIBITI
13. PIBIC-EM

15:00 - 17:30 **Young Scientist Award - Scientific Initiation and PAP Program**

Award Committee:

Prof. Dr. Sandro Rogério de Almeida – (USP)
Dra. Denise Yamamoto – (UNIFESP)
Dr. Pedro Murilo Sales Nunes – (USP)

15:00 - 16:00 **Scientific Initiation Award**
Coordinator: Dr. Durvanei Augusto Maria (Instituto Butantan)

16:00 - 16:30 **Coffee break**

16:30 - 17:30 **PAP Program Award**
Coordinator: Dra. Maria Regina Sandoval (Instituto Butantan)

Thursday 12/05/2013

09:00 - 11:30 **Thematic Session II: Globalization of Technology Development: Challengesfor Production**
Coordinator: Dr. Paulo Lee Ho (Instituto Butantan)

09:00-09:10 **Production at Instituto Butantan:state of art**

09:10 - 09:50 **"Interação entre pesquisa básica e aplicada, na indústria e na academia: minha experiência"**
Dr. Bernardo Boris Vargaftig - Universidade de São Paulo

09:50 - 10:30 **Eliminating dangerous infectious diseases with vaccines: looking back, looking ahead**
Dr. Donald Pinkston Francis - Executive Director at Global Solutions for Infectious Diseases

10:30 - 10:50 **Coffee break**

10:50 - 11:30 **Community Member and Health Care Worker Education, and effective, affordable, available Antivenoms: inseparable elements of a Global Response to Snakebite**
Dr Simon Jensen - Global Snakebite Initiative Limited

11:30 – 13:00 **Lunch**

13:00 – 15:00	**Poster Session II** 7. Cell Biology and Genetics 8. Animal Biology 9. Education and Science Dissemination 10. Others 12. PAP Program
15:00 – 17:30	**Young Scientist Award – Master and Doctoral Degrees** **Award Committee:** Dra. Alessandra Bizerra (USP) Dra. Fernanda Ortis (USP) Dra. Karina Ramalho Bortoluci (UNIFESP)
15:00 – 16:00	**Master Degree Award** Coordinator: Dr. Orlando Garcia Ribeiro Filho (Instituto Butantan)
16:00 – 16:30	**Coffee break**
16:30 – 17:30	**Doctoral Degree Award** Coordinator: Dra. Irina Kerkis (Instituto Butantan)

Friday 12/06/2013

09:00 – 12:00	**Thematic Session III: Initiatives for ScienceDissemination Beyond Frontiers** Coordinator: Dr. Jorge Kalil (Instituto Butantan)

09:00 – 09:10 Challenges and Paths to the Butantan Institute

09:10 – 09:50	**"O papel das sociedades para o avanço da Ciência em um mundo globalizado"** Dra. Helena Bonciani Nader – Universidade Federal de São Paulo/ Presidente da SBPC
09:50 – 10:30	**"A FAPESP e a inserção internacional da ciência feita em São Paulo"** Dr. Walter Colli – FAPESP
10:30 – 10:50	**Coffee break**

10:50 – 11:30	**"Internacionalização da Ciência Brasileira: Programa Ciências sem Fronteiras"** Dr. Glaucius Oliva – CNPq
11:30 – 13:00	**Lunch**
13:00 – 15:00	**Poster Session III** 4. Immunology and Vaccines 5. Microorganisms 6. Biotechnology
15:30 – 15:45	**Inovation Award** **Award Committee:** Emer Suavinho Ferro (ICB/USP) Fernando Queiróz Cunha (FMRP/USP) Ricardo Remer (Remer Consultores Assessoria Comercial LTDA) Neuzeti M. Santos (Instituto Butantan)
15:45 – 16:30	**Young Scientist Award**
16:30	**Closing Session**
17:00	**Cocktail**

1. Venoms and Envenomations

1.01 Analysis of *Tityus serrulatus* venom proteolytic components: determination of biochemical standards and preliminary purification

Cajado Carvalho D[1], Kuniyoshi, AK[1], Kodama RT[1], Tambourgi DV[1], Portaro FCV[1].
[1]Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil

Introduction: As they are the major cause of human poisoning by animals in Brazil, Scorpions have a medical relevance, with *Tityus serrulatus* as the main species. This is due to their easy adaptation to urban centers, its reproductive strategy and the potency of its venom. Several neurotoxic polypeptides that affect ion channels can be found in this venom, and are well known, but little information is available about proteolytic components in the *T. serrulatus* venom (TsV). **Objectives:**Our work aimed to characterize the proteolytic activity of the TsV, assigning new possible biological substrates that could be hydrolyzed during the envenomation. **Methods:** In this first step, the TsV (5 mg/mL) was studied through the use of FRETs substrates in a fluorimeter and biological activity peptides in RP-HPLC C-18. Also, we tested the TsV with class-specific inhibitors and set the ideal buffer conditions.Next, the TsV was fractionated using a 30kDaMolecular Weight Cut-Off (MWCO),retested with selected substrates and subjected to a HPLC purification step using a C-4 RP column, where the peaks collected manually for posterior assays.**Results and Discussion**: To study the TsV proteolytic activity, we determined that the Abz-GGFLRRV-EDDnp FRET substrate is the more susceptible to hydrolysis in fluorimeter, and dynorphin A, in RP-HPLC. The inhibitor test indicated the presence of metalloproteases. The influence of pH, temperature and salt on TsV peptidases was studied and we set up the best in vitro conditions: buffer Tris 50mM, containing NaCl 50mM at pH 8.0. Mass spectrometric analysis revealed that after treatment with TsV, dynorphin exhibits two scissile bonds between the Leu-Arg and Arg-Arg residues, thus producing another biologically active peptide, leu-enkephalin (YGGFL). In an attempt to verify if only one enzyme was responsible for the proteolytic activity, we started a preliminary purification using a 30 kDa MWCO. The obtained solution, named as fraction G, showed the same TsV proteolytic characteristics over the FRET substrate and dynorphin. The fraction G was resolved in a RP-HPLC, resulting in 10 subfractions (G1-G10). Only two showed proteolytic activity (G02 and G10) upon FRET substrate, whereas G02 presented the higher specific activity. Both subfractions were also able to hydrolyze dynorphin and, for our surprise, G10 showed the highest specific activity. More than one metalloprotease is present in the TsV, able to cleave Abz-GGFLRRV-EDDnp and dynorphin. The release of leu-enkephalin by non-physiological mechanisms might elicit unexpected consequences and further studies with pure proteases and its effects in vivo are being carried out by our group.

Supported by FAPESP and INCTTOX

1.02 Analysis Durvenoy's (venom) gland transcriptome of Brazilian false coral snakes *Oxyrhopus guibei*

Campos PF, Oliveira UC, Junqueira-de-Azevedo ILM
Laboratório Especial de Toxinologia Aplicada, CAT, Instituto Butantan, Brazil.

Introduction: Colubrid group comprises the majority of snake species. Despite that fact, these species receive less attention in the field of Toxinology because their bites are, with few exceptions, nonlethal to humans because of their inability to deeply inject the venom. However, few studies on the characterization of these venoms revealed a repertoire of potentially important biomolecules. **Objectives:** In order to identify new toxins and to predict the pharmacological effects and the evolutionary aspects of such venoms, a transcriptome analysis was performed on *Oxyrhopus guibei* venom glands. **Methods:** Venom glands from specimens of *O. guibei* were obtained and total RNA was extracted. Messenger RNA purification was performed using oligodT magnetic beads. Sequencing was performed in a GS Junior 454 Sequencing System following the manufacturer protocols. Sequences were assembled with Newbler Software and RNA-seq quantification performed with CLC Genomics Workbench. Consensus sequences were subjected to a BLAST search and an Automatic annotation using Blast2Go. **Results and Discussion:** Profiling the venom gland transcriptomes from 1472 contigs assembled, it was possible to identify transcripts with high degree of similarity to common snake toxin families: snake metalloproteinase (SVMP – PI and PIII), C-type lectin-like, CRISP, Kunitz-type inhibitor, Phospholipase A_2 inhibitor, Serine protease inhibitor as observed in the transcriptome of other colubrids. About 17% of all transcripts code for toxins, among them, SVMP represents the major protein type. Furthermore, components involved in cellular process were observed and 41% of the transcripts remained unidentified. This data shows that some snakes belonging to Colubridae family are still poorly known concerning venom composition. The next-generation sequencing analysis showed trends governing the formulation of the venom arsenal. Currently, we are investigating the venom proteome in order to get hints on the translation efficiency of toxin-coding transcripts, contributing thereby to a more accurate interpretation of the transcriptome.

Supported by CAPES and FAPESP

1.03 Comparative study of enzymatic properties and antigenic cross-reactivity of *Tityus serrulatus* (Scorpiones - Buthidae) venom obtained from specimens of different Brazilian regions

Candido DM[1,3], Preto GEO[2,4], Távora BCLF[2,3], Barbaro KC[2]
[1]Laboratório de Artrópodes, [2]Laboratório de Imunopatologia, [3]Programa de Pós-Graduação em Toxinologia, [4]PAP, Instituto Butantan, Brazil

Introduction: The scorpion envenomation is a major public health problem in Brazil. It was notified around 59,570 accidents in 2011, with 87 deaths. The mainspecie thatcauseserious accidentsand evendeath is*Tityusserrulatus*. The envenomations of *T. serrulatus* is characterized mainly by intense pain and neurotoxic effects. The serumtherapy is recommended by Ministry of Health as treatment of moderate and severe cases of scorpions envenomation. Instituto Butantan produces the antivenom serum with *T. serrulatus*scorpions which are kept in captivity.Data from the Ministryof Health showthat there aredifferencesbetween the severityof symptomsof envenomation by *T. serrulatus*from different regions ofBrazil. For instance, the incidence of *T. serrulatus* has increased in Distrito Federal and Bahia State, however there are few reports of severe scorpionism in these regions **Objectives:** This study aims to evaluate if there are differences of enzymatic properties and antigenic cross-reactivity between the venom obtained from specimens of *T. serrulatus* captured from five different Brazilian states. **Methods:** Silver stained SDS-PAGE (12%) was used to compare the protein profile of from *T. serrulatus* venoms (20 µg) obtained from specimens collected of São Paulo, Minas Gerais, Paraná, Distrito Federal and Bahia states. Zymography was used to detect proteolytic and hyaluronidase activities in these samples (100 µg) using fibrinogen (0.5 mg/mL), casein (2 mg/mL), gelatin (2 mg/mL) and hyaluronic acid (170 µg/mL) as substrate. To detect antigenic cross-reactivity by ELISA was used antiscorpion serum (ASS) produced by Ezequiel Dias Foundation.**Results and Discussion:** By SDS-PAGE (12% acrylamide) all the samples had similar electrophoretic profiles with proteins distributed mainly in 17-25 kDa and 48-52 kDa regions. None of venoms obtained from scorpions from São Paulo, Minas Gerais, Paraná, Distrito Federal and Bahia states showed activity on substrates gelatin, casein or fibrinogen. Only hyaluronidase activity was observed using 100 µg of all samples (bands around 37 kDa). Antigenic cross-reactivity using antiscorpionic serum (ASS) was detected by ELISA in all samples studied (titer 256,000). No significant differences between the samples obtained of specimens of the five states studied were observed.Comparative biological studies (lethality and nociception) will be performed to verify if there are differences in toxic activities.

Supported by CAPES, FAPESP, PAP program and INCTTOX

1.04 Antiproliferative effects of jararhagin toxin on human leukemic cell

Conceição TO, Silva MGL, Laiso RAN, Maria DA
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Introduction: Jararhagin is a type metalloprotease disintegrin isolated from the venom of Bothrops jararaca, comprising metalloproteinase domain, disintegrin and cysteine-rich. In vitro studies have shown that treatment with the toxin reduces cell viability and have inhibitory effects on the adhesion to the substrate of tumor cells, fibroblasts and endothelial cells. This study evaluated the antitumor effects, the cell changes and formation of lipid peroxid radical the line of human leukemia K562 cells after treatment with different concentrations of jararhagin.**Objectives:**Evaluated effects of toxin antiproliferative and pro-apoptotic in K-562, human leukemic cell.**Methods:**The tumor cells were treated with different concentrations jararhagin and cytotoxicity assessed by MTT colorimetric assay. Jararhagin treatment resulted in the significant increase in the formation of radicals peroxid. The analysis of the cellular modifications obtained by confocal laser scanning microscopy showed morphological changes such as the formation of apoptotic bodies at low concentrations of the toxin and cells undergoing necrosis at high concentrations. **Results and Discussion:** According to the evaluation of the MTT, treatment with jararhagin showed cytotoxicity in leukemia cell line with IC50% 1.14 µg/mL. Changes in morphology, the kinetics of cell growth and suggests that the toxin induces cell death by apoptosis in low concentrations and proportions in the production of free radical. These results point to the potential use of this toxin as a tool for applied research in the clinical field.

Supported by CNPq and FAPESP.

1.05 Immunochemical characterization of *Bothrops lanceolatus* venom: a "pathway" to complement cascade activation

Delafontaine M[1, 2], Paixão-Cavalcante D[1], Portaro FCV[1], Mathieu L[2], Blomet J[2], Tambourgi DV[1]
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[2]Prevor Laboratory, Moulin de Verville, 95760 Valmondois, France

Introduction:*Bothrops lanceolatus*, commonly named "Fer-de-Lance", is the endemic snake of the French Caribbean Island Martinique, where it is responsible for about 20-30 bites per year. Envenomations by *B. lanceolatus* cause systemic thrombotic syndrome and important local inflammation, involving oedema, pain and haemorrhage from fang punctures. It has been shown that several bothropic venoms from Central and South America activate complement system and that metallo and/or serine proteinases are involved in this process. **Objectives:**In this study we have immunochemically characterized the venom of *B. lanceolatus* with the aim to investigate its complement cascade activation potential. **Methods:**We used electrophoretic separation, zymography, colorimetric or fluorimetric (FRET) enzymatic assays, in addition to immunochemical assays to characterize *B. lanceolatus* venom. **Results and Discussion:**As it had been shown by other authors, *B. lanceolatus* venom has phospholipase, gelatinolytic and strong fibrinogenolytic activities. Surprisingly, this venom is deprived of hyaluronidase activity. Western blot analysis using lectins highlighted the presence of glycosylated proteins, which may activate complement cascade via the lectin pathway. This activation was confirmed by ELISA assay testing the binding of C4. Haemolytic assays showed that *B. lanceolatus* venom activates the complement cascade by both alternative and classical pathways. Further, the components of *B. lanceolatus* venom share antigenic similarities with South American *Bothrops* species, since the antibothropic serum raised against Brazilian snakes from Butantan Institute (São Paulo, Brazil) cross-reacted with *B. lanceolatus* venom in Western blot and ELISA assays. The antibothropic serum partially inhibited the cleavages of two FRET peptidic substrates by the venom of *B.lanceolatus*: peptides Abz-RPPGFSPFRQ-EDDnp and Abz-FRSSRQ-EDDnp that are preferentially cleaved by metalloproteinases or serine proteases, respectively.All together these data indicate that *B. lanceolatus* activatesthe complement cascade via classical, alternative and lectin pathways, fact that could play an important role in the local inflammatory reaction and haemostasis disturbance caused by *B. lanceolatus* venom, via a strong release of anaphylatoxins. More investigations are still needed to identify the proteases involved and their mechanisms of action on complement, however the present data suggest that the mechanism could be similar to what was described with other bothropic venoms.

Supported by Prevor Laboratory (France)

1.06 Evaluation ofcytokine levels in embryos of rats treated with *Tityus bahiensis*scorpion venom during pregnancy

Dorce ALC[1,2], Frare EO[1], Val de Paulo MEF[1], Santos EMR[1], Dorce VAC[1], Nencioni ALA[1]

[1]Laboratory of Pharmacology from Butantan Institute – São Paulo/Brazil;

[2]Post-graduation Program in Sciences of the Center for Disease Control from the Health Ministry of the State of São Paulo, Brazil.

Introduction: The inoculation of scorpion venom produces an injury in the tissue that can induce a systemic inflammatory response, with consequent release of cytokines. Cytokines play a critical role in pregnancy and embryonic development by participating in processes such as implantation of blastocyst formation and embryo development. The work performed by our group with the venoms showed that it causes fetal resorption and changes in the organ development and in the physical and behavioral development of the pups when mothers receive it during pregnancy. **Objective:** Verify the possible changes in the cytokine levels of IL-1α, IL-1β, TNF-α, IL-6, IL-10, and INF-γ in embryos after the treatment of pregnant females with the *T. bahiensis* scorpion venom. **Methods:** The procedures were approved by the Ethics Committee on Animal Use of the Butantan Institute under protocol number 513/08. For the study, embryos and their placentas were used, which were obtained from the crossing of male and female Wistar rats approximately 90 days old (260-300g). To evaluate the cytokine levels, pregnant females were injected with saline (control group) at a dose of 1ml/kg, lipopolysaccharides (LPS) at a dose of 100μg/kg (positive control) or crude venom of *T. bahiensis* scorpion at a dose of 2.5 mg/kg (experimental groups) on the 10th or 16th gestational day. The pups were removed by laparotomy 6 and 24 hours after treating the mother. The samples were macerated by a tissue homogenizer and centrifuged at 10,000 rpm/10min at 4°C. Tissues were placed in protease inhibitor cocktail. Cytokine levels were determined by enzyme immunoassays. **Results and Discussion:** After 6 hours, in GD 10 no alterations in cytokine levels were observed. In GD16, an increase on the IL-1α level (C 56.95±13.70; LPS 64.51±9.86; E 209.6±27.01) was observed; the other cytokines were not altered. After 12 hours, no alteration was observed in the cytokine levels. After 24 hours, a decrease in the INF-y level (C 1045.0±153.3; LPS 258.4±57.4; E 273.2±34.97) was observed in GD10. In GD16, a decrease in the IL-10 (C 319.0±27.21; LPS 136.6±27.81; E 168.6±33.29), TNF-α (C 1022.0±268.9; LPS 318.1±178.8; E 110.1±69.23) and INF-y (C 297.7±82.08; LPS 158.9±60.35; E 17.53±4.56) levels was observed. The moderate maternal envenomation by the *T. bahiensis* scorpion venom causes some alterations in cytokine levels after the treatment.

Supported by CAPES and FAPESP(11/10222-5)

1.07 Study of the modulatory potential of crotoxin and its subunits isolated from *Crotalusdurissusterrificus* venom on dendritic cells

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Introduction: It has been shown that products of pathogens as well as their secreted antigens are able to modulate the immune system. The *Crotalusdurissusterrificus*rattlesnake venom (*C.d.terrificus*) and its main toxin, crotoxin (CTX) have suppressive effect on immune system. It has been shown in distinct experimental models that the subunit CA (crotapotin) or CB (phospholipase A2) isolated from CTX exerts this immunomodulatory action. The dendritic cells (DCs) are involved in the generation of the adaptive immune response or in maintenance of the immune tolerance. **Objective:**The aim of this work was to study the modulatory effect of CTX as well as of its subunits on functional activity of DCs *in vitro*.**Methods:**The CTX, CA and CB subunits were purified, analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and the endotoxin (LPS) content eliminated in polymyxincolumn.Immature DCs (iDCs) were derived from BALB/c mice bone marrow in RPMI medium containing only GM-CSF or GM-CSF/IL-4 for 7 days. After this, the cellular phenotype was analyzed by flow cytometry. In other experiments, these cells were incubated with CTX, CA or CB in the presence or not of LPS for 18 hand subsequently the supernatants also were collected for the cytokines determination. **Results and Discussion:**The flow cytometry analyses showed 85% of CD11c^{+} cells in the culture differentiated with GM -CSF + IL-4 compared to 37% obtained with the protocol only GM-CSF. The immature CD11c^{+} cells express Toll like receptors 1, 2, 4 and C-type lectin receptors (MR and DC-SIGN). Higher expression of MHC class II (MHC-II) and costimulatory molecules expression were observed in DCs generated with GM-CSF/ IL-4 and incubated with LPS when compared with those observed in DCs differentiated with GM-CSF. The CTX, CA or CB at two different concentrations did not induce increase of MHC-II and costimulatory molecules in DCs. CTX and CB subunit also inhibit the expression of costimulatory and MHC-II molecules in DCs incubated with LPS. However, the CA was not able to inhibit the maturation of DCs by LPS. The cytokines assays showed high secretion of TNF-α, IL-1β, IL-6 and IL-10 in DCs cultures incubated with LPS. In addition, we found that CTX was able to inhibit the secretion of IL-10 and IL-1β by DCs incubated with LPS. These results demonstrate that CTX and CB subunit but not CA exert modulator effect on DCs activation when incubated with a TLR4ligand as the LPS.

Supported by FAPESP (2011/23735-0) and CAPES

1.08 Nephrotoxicity of *Apis mellifera* bee venom in mice

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Introduction: Bee sting is a significant public health problem. Bee venom (BV) is a complex mixture of toxins. Some reports exist about the occurrence of rhabdomyolysis and hemolysis in patients envenomed by BV. The kidney is highly vulnerable to the venoms' toxicity, since it is well vascularized. Although it is known that BV can cause renal dysfunction, the characteristics of its nephrotoxicity are still not known.**Objectives:**To contribute for the knowledge of renal dysfunction resulting from the envenomation by BV.**Methods:**Hematocrit, and osmolality, protein, alanine aminopeptidase (AAP), creatinine, urea and uric acid in urine and plasma, and oxidative stress (GSSG/GSH and malondialdehyde [MDA]) in the renal medulla and cortex were measured inadult, male Swiss mice, 18-20g, submitted to a single injection, in bolus, of BV (2.5 mg/kg at maximum volume 50μL/animal, subcutaneously on the dorsum) (envenomed - E) or the same volume of 0.9% NaCl (control - C). The experimental procedures are in accordance with the protocol 858/11 approved by the Ethics Committee on Animal Use of Butantan Institute. Data were expressed as mean±SEM, and statistically analyzed (E versus C) by unpaired, two-tailed Student's t test, $p<0.05$, using the GraphPad Prism^{tm} software package. **Resultsand Discussion:** Among the parameters evaluated the following were different between C and E: Urinary osmolality (mOsm/kg): C, 880±5.00 (pool of 5 animals in triplicate); E, 771.67±1.66 (pool of 10 animals in triplicate), and medullar MDA (nmol/mg protein . 10^{-6}), C, 0.68±0.02 (N=5); E, 1.06±0.09 (N=9).Oxidative stress is a critical factor in the development of acute renal failure. BV dose used here mimics the sting, and it is around the intraperitoneal and intravenous LD50 (2.8-3.8 mg / kg) of the European and Africanized BV, being 5 times higher than that reported to cause the reduction in the glomerular filtration rate and renal blood flow, predominantly in the cortex, when infused in the jugular vein of rats. The trend of GSSG/GSH index to be increased and the significant increase of MDA detected in the renal medulla of envenomed suggest renal damage, mainly in the tubules, the most abundant structures in this portion. These damages should be aggravated by urinary hypo-osmolality that accompanies this increase, indicating the tendency of deleterious effects in blood electrolytes. Taken together these changes must be related to an initial stage of kidney injury, allowing to infer that there is an imminent risk of kidney damage in the accidents by bee stings, which depends on individual susceptibility and inoculated dose, both which still are imponderable factors in this envenomation, and thus suggesting that preventive administration of nephroprotector agents may be useful in these accidents.

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1.09 Action mechanisms of sphingomyelinase D from *Loxosceles* spider venom on human epidermal cells: implications for dermonecrosis development

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Introduction:*Loxosceles* spider venoms consist of a mixture of proteins with enzymatic or toxic activity including sphingomyelinases D (SMases D), a PLD considered as the main toxic venom component responsible for the establishment of local and systemic effects. In *Loxosceles* spider accidents, the serum therapy has been widely used; however, there is no consensus about its effectiveness, mainly for the control of the local lesions. Studies of our group have shown that in the cutaneous loxoscelism the binding of SMases D to keratinocytes with subsequent cell death, involves at least two mechanisms: the expression/secretion of matrix metalloproteases (MMPs) and activation of surface metalloproteinases of the Adamlisin family, which lead to the cleavage of molecules on the surface of these cells. Beyond that, as a multifactorial process,the local production of inflammatory mediators may contribute to the development of loxoscelic lesion. **Objectives:** This study aims to evaluate the effect of the SMase D on human keratinocytes, regarding to the production and secretion of cytokines, reactive oxygen and nitrogen species, expression of apoptosis receptors and cell signaling possibly involved in the development of local lesions.**Methods:**HaCat cells were treated with SMase D for two hours, the apoptosis receptors expression and production of reactive oxygen and nitrogen species was analyzed by flow cytometry. The cytokine secretion was assessed by ELISA, in the supernatant of keratinocyte cultures treated for 72 hours. The MAPK signaling pathway was evaluated using the commercial ELISA kit of pERK1/2 detection in extracts of cells treated with SMase D/*L. laeta* venom for 30 and 60 minutes. **Results and Discussion:**The results revealed that the SMase D and *L. laeta* venom were able to induce the activation of MAPK ERK1/2 signaling pathway. The apoptosis event in keratinocytes, induced by SMases, does not occur by the action of death receptors such as Fas/FasL and TNF-RI. TNF-RI expression was reduced in cells treated with the toxin, possibly by cleavage or endocytosis. The toxin is able to induce oxidative stress with the production of superoxide anion in these cells, as well as cytokines such as IL-1β, IL-6, TNF-α and TGF-β1. The results indicate that the effects caused by SMase D in keratinocytes may contribute to development and difficult to heal the local lesions observed in *Loxosceles* spider envenomation and these should be considered in the development of complementary therapies.

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1.10 The venom of four African snakes species analysis: studying the low molecular mass constituents and their biological activities.

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Introduction: Snake venom poisoning is a public health issue for many countries. Studies show that the number of accidentssurpasses the number of fatalities from several other tropical diseases. In addition, snake bites only joined the list of neglected tropical diseases recently, in April 2009, showing that it was not seen as an important public health issue by WHO. In the sub Saharan Africa is annually registered approximately 300,000 cases of accidents by snakes which results in 32,000 deaths and a large number of victims with permanent local tissue damage and chronic disabilities. *Bitis nasicornis*, *B. rhinoceros Naja mossambica* and *Dendroaspis polylepis* are some of the highest medical important venomous snakes and these venoms are rich sources for proteolytic enzymes. Some studies have demonstrated that *Bitis* sppvenom act on macromolecular substrates causing an imbalance of the prey's hemostatic system. There are only few studies about the effects of snake venoms small molecules, like peptides. **Objectives:** Study the low molecular mass constituents (<10 kDa) from the venoms of *B. nasicornis,B. rhinoceros,Naja mossambica* and *Dendroaspis polylepis* seeking modulators of human peptidases. **Methods:** The*peptide pools* fromall four species venoms were obtained by molecular weight cut-off and incubated with ACE (angiotensin-converting enzyme) using Angiotensin I as substrate.The inhibition of Angiotensin I hydrolysis was detected by HPLC analysis and the composition of the two most potent *peptide pools,*from *B. nasicronis* and *B. rhinoceros,* were analyzed using a C-18 column in HPLC. The observed peaks were collected and incubated with Abz-FRRK(2,4-dinitrophenol)P-OH and ACE in order to obtain purified peptides.**Results and Discussion:**The most significant inhibition of ACE was observed when incubated with *peptide pools* from *Bitis nasicornis* and *Bitis rhinocerus*, followed by *Dendroaspis polylepis* (KENYA) and *Naja mossambica*. The HPLC analysis in C-18 column of *peptide pools* from *Bitis nasicornis* and *Bitis rhinoceros* showed 11 and 9 peaks respectively. The fluorimetric assay exhibited that the peaks with significant inhibition were the numbers 6 with 21% inhibition, 7 (54%), 8 (71%), 9 (78%), 10 (65%) and 11 (40%) from *Bitis nasicornis peptide pool*, and the peaks numbered 6 with 75% inhibition, 7(58%) and 8 (59%)from *Bitis rhinocerus peptide pool*. The results are consistent with the symptoms of the victims of *Bitis*spp, who display a severe hypotension. This work is in progress to identify and to sequence these molecules.

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1.11 Pancreatic peptides as substrates for serine peptidases from the venom of *Bothrops jararaca* (BjV)

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Introduction: Snake venom poisoning is a public health issue for many tropical countries. Studies show that the number of accidents surpasses the number of fatalities from several neglected tropical diseases. For this reason, snake bites joined the list of neglected tropical diseases recently, in April 2009, showing that it was not seen as an important public health issue by WHO. In Brazil, *Bothrops* spp poisoning is responsible for 75% of snake bites and *Bothrop'*svenoms are rich sources for proteolytic enzymes (65% of the composition). Most studies have demonstrated that BjV act on macromolecular substrates causing an imbalance of the prey's hemostatic system. There are only a few studies about the effects of snake venoms upon small molecules, like peptides **Objectives:** Search for new bioactive peptidic substrates for the metallo- and serine peptidases present in the *B. jararaca* venom (BjV) that could be related with envenomation symptoms. **Methods:** The BjV was incubated with somatostatin and pancreatic polypeptide (PP) using the site-direct inhibitors PMSF (3 mM) and 1,10- phenanthroline (3 mM). The hydrolysis of these peptides was detected by HPLC analysis. **Results and Discussion:** Initially, we observed the hydrolysis of the pancreatic polypeptide by the BjV, and this activity was partially inhibited by 1,10-phenanthroline and fully neutralized by PMSF, indicating that serinepeptidases are responsible for the primary cleavage of this peptide. We also incubated another peptide, somatostatin, with BjV and it was also hydrolyzed. The inhibition assay indicated the same result of PP, that serinpeptidases were responsible for this activity. Both peptides are related to the mammal's energy homeostasis regulation, with PP being secreted in situation of hypoglicemia and exercise, both related with the situation of the prey/victim. Somatostatin is responsible for regulating the secretion of PP, insulin and glucagon, therefore indirectly influencing glycemic level. Taking into account that BjV can hydrolyze insulin B chain, we suppose that molecules of this venom were undergone natural selection in order to destabilize the prey's energy homeostasis, as well as all other known systems affected and already described.

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1.12 A new recombinant disintegrin from *Bothrops neuwiedi* snake

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Introduction: Disintegrins are a family of low molecular weight and cysteine-rich proteins isolated from viper venom. These proteins bind to integrin receptors using a conserved binding motif sequence containing RGD or similar motifs. As a consequence, disintegrins can inhibit platelet aggregation and inhibit cell migration, proliferation and initiate apoptosis in cancer cell lines. Dis-BnMPIIx is a disintegrin cloned and sequenced from *Bothrops neuwiedi* venom gland transcripts, it has a classic sequence RGD and two extra cysteines. **Objectives:** In this study, our objective is to clone and express the Dis-BnMPIIx in bacterial system and analyze their biological activity. **Methods:** The cDNA of Dis-BnMPIIx was amplified by PCR, digested by specific endonucleases (*BamHI* and *HindIII*) and cloned into, pSMT3 vector. The sequencing of all inserts showed that no mutations were introduced during the cloning procedures. These vectors were transformed into *E. coli* C43 (DE3) strain and expression was carried out at 37ºC by induction with IPTG. After the expression the protein was released from the SUMOfusion protein by cleavage with *Ulp*1 enzyme overnight at 30ºC. Free disintegrin was purified by IMAC (immobilized metal affinity chromatography) and tested by SDS-PAGE. To test platelet aggregation, human blood was collected in a 3.8% sodium citrate (1:9) from healthy donors. The blood was centrifuged and the platelet-rich plasma (PRP) was submitted to an ADP-induced platelet aggregation assay. **Results and Discussion:** The SDS-PAGE analysis showed that recombinant Dis-BnMPIIx was expressed in *E. coli* C43 (DE3) at 37ºC in soluble form in fusion with SUMO. After cleavage with *Ulp*1 enzyme, the isolated disintegrin showed full activity on the inhibition of platelet aggregation in a dose of 2.3 μM and partial inhibition in a dose of 1.17 μM.The expression of soluble Dis-BnMPIIx wassuccessful in pSMT3 vector. This recombinant protein may be used in further studies of inhibition assays of cell systems dependent on integrins.

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1.13 The accessory gland of *Bothrops jararaca* snake secretes toxins

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Introduction: The venom apparatus of viperid snakes is composed by four distinct parts: main venom gland, primary duct, accessory glandand secondary duct. Morphological analysis the accessory gland at different steps after venom extraction showed that this gland has a long cycle of production and secretion that is not synchronized with the secretory cycle of the main venom gland, and a massive exocytosis occurring 4 days after venom extraction. Thus, it seems that its secretion do not contribute significantly to whole venom.**Objectives:** The aim of this study was to identify the proteins that are present in accessory gland at different steps after venom extraction.**Methods:** Accessory glands were obtained from female *Bothrops jararaca* snake in which venom was not manually extracted (0d) and from snakes in which venom was extracted 4 and 7 days (4d and 7d), before they were killed by decapitation (N=3 for each group), in order to observe changes that occur during the secretory cycle. Extracts of these glands were prepared and the proteins were analyzed by SDS-PAGE electrophoresis. The bands were digested with trypsin and their protein content identified by ESI-LTQ XL/Orbitrap MS. All MS data were analyzed using PEAKS Studio 5.3 and searches were made using NCBInr and snake venom BSI databases. All information about proteins identified is collected on UniProt.**Results and Discussion:** For the first time, we identified the presence of toxins in the accessory gland. Toxins such as C- type lectins (CTL), metalloproteases (SVMP), serine proteases (SVSP), L-amino acid oxidase (LAAO), phospholipases A_2 (PLA_2) and disintegrin were present in the accessory gland. Furthermore, glutaminyl cyclase (GC) and natriuretic peptide (NP), a secreted protein, were also identified in the accessory gland. Two endogenous metalloproteinase inhibitors (Bj46a and pEKW) and PLA_2 inhibitor (PLI) were also present in this gland. Similar to the main venom gland, we identified toxins in the accessory gland in all group studied. The number of SVSP, PLA_2, PLI, GC, PN, pEKW and disintegrin species was higher 0d group than in other groups, and SVMP, LAAO, Bj46a was higher after venom extraction (4d or 7d group). Therefore, the synthesis of new toxins is asynchronous like in main venom gland. In conclusion, these results showed that the secretion of the accessory gland contain toxins that could contribute to the whole venom, although the exocytosis occurs late. This study will give us new insides to understand the mechanism of venom production of venom gland apparatus and open new avenues for toxins studies.

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1.14 Neurological development of the offspring of female rats treated with scorpion venom *Tityus bahiensis* during lactation.

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Introduction:The*T.serrulatus* and *T.bahiensis* are the main causes of envenomation in humans in Brazil. Several studies show the effects of the venom in humans, but there are no studies that report its effects on postnatal development of offspring of mothers who received the venom during the lactation period (PN). **Objectives:** Study the effects on the physical, behavioral and reflex development of the offspring of mothers injected with the venom of the scorpion *T.bahiensis* at PN2, 10 or 16 of lactation. **Methods:**Females and their litters were divided into 6 groups, 3 control groups injected with 1ml/Kg of saline and 3 experimental groups injected with venom in a dose of 2.5mg/Kg. The rat pups (neonatal period) were evaluated according to their physical and neurobehavioral development. The pups in the adulthood were evaluated according their behavioral development. The same pups in both stages of development were evaluated to neuronal integrity in the CA1, CA3 and CA4 of the hippocampus. For determination of IL-1α,IL-1β,IL-6,IL-10,INF-γ,TNF-α levels the rats and their pups were treated on the PN10 or 16 of lactation 1ml/kg saline or 100μg/Kg LPS or 2.5mg/kg venom. **Results and Discussion:**On the physical development of the offspring, female and male animals presented alterations related to the gender on the parameters observed, such as a delay on ear unfolding, ear opening and tooth rupture. The development parameters during the neonatal stage also presented changes, such as in the palmar grasp tests, righting reflex, negative geotaxis and general activity in the activity box. These alterations could indicate changes in the maturity of the structures in the central nervous system (CNS) involved with the animal's motor and spatial ability. In adulthood, behavioral changes were observed, such as low anxiety, learning and memory deficit, and reduction in the motor ability. We observed an increase in the number of viable cells in the CA1and CA4 region, especially in puppies the 2nd day of lactation. Levels of cytokines, an increase in the concentration of IFN-γ in group PN10 compared to the control group, and the PN16 the increase was related to the positive control group. The other cytokines were not altered. With these results, we conclude that the venom, directly or indirectly, is able to cause physical, reflexology and behavior changes in offspring of mothers injected during the lactation period. These changes may be related to interference in hippocampal neurogenesis mediated immune factors present in this microenvironment. Although such changes do not show intensity or frequency, these may be due to interference during critical periods of maturation of the CNS.

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1.15 Hemorrhagic activity of HF3, a snake venom metalloproteinase: insights from the peptidomic analysis of muscle cells

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Introduction: Manifestations of local tissue damage, such as hemorrhage and myonecrosis, are among the most dramatic effects of envenomation by snake species of the Viperidae family. Snake venom metalloproteinases (SVMPs) of the P-III class are main players of the hemorrhagic effect due to their activities in blood vessel disruption and inhibition of platelet aggregation. Hemorrhagic Factor 3 (HF3), a P-III class SVMP from *Bothrops jararaca*,shows a minimum hemorrhagic dose of 240 fmol on the rabbit skin. A recombinant protein composed of non-catalytic domains of HF3 (disintegrin-like and cysteine-rich domains; DC-HF3) inhibited collagen-induced platelet-aggregation and increased leukocyte rolling in the microcirculation. **Objectives:**To evaluate the effect of HF3 on myoblast cells by the peptidomic analysis of culture supernatant. **Methods:**In this study, differentiated murine C2C12 skeletal muscle cells were incubated with native HF3 (50 nM) and the DC-HF3 protein (1 uM), for 2h at 37°C. The peptide fraction of the culture media was concentrated by solid phase extraction and peptidomic analysis was carried out by liquid-chromatography coupled to high resolution tandem mass spectrometry (LC-MS/MS) using a LTQ Orbitrap Velos. Spectra were analyzed using the software package MaxQuant and the SwissProt database. **Results and Discussion:** The peptidomeof the culture medium of cells treated with DC-HF3 showed low complexity, however, treatment of C2C12 cells with HF3 revealed expressive proteolysis and pointed out potential new substrates of HF3 including collagen XVIII, transgelin, and nestin. These peptides were likely generated by proteolysis by HF3, however, activation of cell proteases/networks cannot be ruled out. This work shows for the first time the targets of HF3 on muscle cells, and can contribute to future studies aimed at explaining the inflammation process and hemorrhage caused by HF3.

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1.16 Role of Phospholipases A_2 in biogenesis of lipid bodies in leukocytes recruited by distinct species of Bothropssnakes venoms

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Introduction: Bothrops genus snake venoms contain a complex mixture of components, some of them with the ability to induce inflammation. Phospholipases A_2 (PLA_2) are major components of these venoms and exhibit inflammatory activities stimulating leukocyte functions including lipid bodies (LBs) formation. These inducible lipid-rich cytosolic inclusions are dependent on the scaffold protein perilipin 2 (PLIN2) and increase during inflammatory processes. **Objectives:** To investigate the effects of crude venoms of *B. jararaca* (BjV) and *B. moojeni* (BmV) in mice peritoneal leukocytes, evaluating: i) LBs formation, ii) protein expression and localization of PLIN2 and iii) contribution of the venom PLA_2 component to LB formation. **Methods**: Swiss male mice were used (Butantan Institute Ethical Committee 729/10). These animals received intraperitoneal (i.p.) injection of an inflammatory dose of each venom sp (0.250 mg/g) previously incubated or not with p-bromophenacylbromide (p-Bpb) (0.1 mM), a PLA_2 inhibitor, for 24h or with vehicle (control). After 6 h, inflammatory exudates were harvested to determine: a) total number of leukocytes in Neubauer chamber, b) leukocyte subtypes in Hema3 stained cell smears and c) LBs formation in macrophages (MΦs) stained with 1% osmium tetroxide followed by phase contrast microscopy counting. PLIN2 expression and subcellular distribution was evaluated by W. blotting and immunofluorescence assays, respectively. **ResultsandDiscussion:**Intraperitoneal injection of either BjV or BmV significantly increased LBs numbers (3.24 ± 0.33 or 5.47 ± 2.3, respectively) in leukocytes collected 6 h after their injections in comparison with controls (0.95 ± 0.07), without statistical difference among venoms. Moreover, BmV significantly increased PLIN2 protein expression at 6 h and BjV at 12 h after injection as compared with controls. MΦs from animals injected with BjV or BmV showed a punctual PLIN2 localization, co-localized to LBs/neutral lipids. Injection of p-Bpb-BjV did not cause LBs formation in MΦs compared to BjV injected group. In MΦ from mice that receive p-Bpb-BmV the number of LBs was reduced in 42% as compared with BmV. These data indicate the ability of distinct species of *Bothrops* snake venoms to induce LBs formation and PLIN2 protein expression and recruitment in MΦ. Moreover, data indicate that PLA_2s play a important role in LBs formation induced by the whole venom of *B. jararaca* and *B. moojeni* snakes, being essencial for *B. jararaca*-induced effect.

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1.17 Comparative analysis of platelets stimulated with thrombin, TRAP and PA-BJ: a proteomics approach

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Introduction: Platelets are anucleate fragments derived from precursor megakaryocytes and play essential roles in normal thrombus formation, thrombosis, inflammation and atherosclerosis. Following activation, platelets undergo release of a-granules, dense granules and lysosomes.**Objectives:**Toanalyze the secretome of platelets after activation with the agonists thrombin (2 nM), thrombin receptor activating peptide (TRAP; 10 μM)and PA-BJ (100 nM), a serine proteinase isolated from *Bothrops jararaca* venom that induces platelet-aggregation by cleavage of thrombin receptor PAR1.**Methods:**After treatment with each agonist, secreted proteins were separated from peptides by acetone precipitation and both fractions (proteins and peptides) were evaluated by liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-MS/MS) and bioinformatics analysis.**Results and Discussion:**Treatment of platelets with all agonists resulted in the identification of components of inflammatory and hemostatic processes, including fibrinogen, von Willebrand factor, thrombospondin-1, coagulation factor XIII, multimerin-1, BM-40, platelet basic protein and platelet factor 4, among the proteins present in the secretome. The peptidomic analysis showed partial degradation of some of these proteins in the secretome of platelets activated by PA-BJ and thrombin. Notably, peptides from fibrinogen, a major protein involved in hemostasis, were abundant in the secretome of activated by PA-BJ and thrombin. Although a number of peptides seemed to be generated by direct proteolytic activity, indirect proteolysis, triggered by lysosomal enzymes, cannot be ruled out. Our results provide insights into the possible participation of peptides generated upon activation of platelets by serine proteinases in the modulation of thrombus formation.

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1.18 Biological activities of the *Bothrops atrox* venom from Santarém region, in Western Pará

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Introduction: *Bothrops atrox*(Ba)snakeis responsible for most of the snakebitesin the Brazilian Amazon region and its venom induces a variety of local and systemic effects.
However,thisvenomisnotincludedinthepoolofimmunization*Bothrops*antivenom (BA). It is also known that venoms from the same species of snake may present geographical, ontogenetic and sexual variations. **Objectives:**To characterizesomebiologicalactivitiesof*Bothrops atrox*venom (Bav)andverifiedthecapabilityof BAto neutralize the coagulant activity.**Methods:**Lyophilized crude venomfrom adult,males(3)andfemales(3)Basnakes(n=6),capturedat FlorestaNacional(FLONA)doTapajós,Santarémregion(Belterra),Pará,wasused toevaluate the following activities: coagulant,phospholipase,fibrino(geno)lytic, plateletaggregation, desfibrinogenantingandedematogenic. It was also measured the capability of BA to neutralizethecoagulant activity.Adult*B.jararaca*venom (Bjv)from Instituto Butantan was used to comparewith Bav mainly *invitro*assays.**Results and Discussion:** Thelower minimumcoagulant doseof Bavon bovine plasma (MCD-P=10.87 ± 0.42 μg/mL) and fibrinogen (MCD-F=17.68 ± 1.67 μg/mL), whencompared to Bjv (MCD-P=35.89±0.22μg/mL;MCD-F=68.23μg/mL)indicatethatBav is morecoagulant than Bjv.However, BA wellneutralizedcoagulantactivityofBav (626.80 μL antivenom/mg venom)and Bjv (1400 μL antivenom/mg venom), for 2x DMC-P. In relation to the activation of procoagulantfactors byBav,higheractivityonfactorII(636.71 ± 25.76 μmol *p*-nitroaniline/min/mg venom) was found in comparisontofactorX(182.15 ± 4.87 *p*-nitroaniline/min/mg venom).TheBav wasabletohydrolyzephospholipids(1,549.37 U/mg venom) and directlydegrades fibrinondose-dependentmannersimilarto Bjv.The Bav inducedaquickdegradationofhumanfibrinogenAαchain,aslow hydrolysisoftheBβchainandtheγchain was not degradated,differinginrelationto thefibrinogenolyticactivityof Bjv ontheBβchainwhichitwaslower. Unlike Bjv, platelet aggregation was not observed in Bav. It wasalso observedthat Bav showedhighdesfibrinogenanting and edematogenic activities. Our data indicate variability between the biological activities of Bav from FLONA and Bjv from Instituto Butantan,aswellasthoseof Bafromothergeographicalorigins.Thisvariabilitymayinfluenceontheseverityofclinical manifestationsobservedonvictimsofsnake bites andthenecessity or not ofmore effectiveantivenoms.

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1.19 Cloning and characterization of human anti-crotoxin (scFv): Expression of three mutants using affinity suggested *in silico*

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Introduction: Crotoxin is the main toxic component of *Crotalus durissus terrificus*. It is a heterodimeric β-neurotoxin that consists of a weakly toxic basic phospholipase A_2 and a non-enzymatic, non-toxic acidic component (crotapotin). In a previous work, crotoxin (CTX) was used to select human neutralizing recombinant scFvs by phage display technology from a naive library of more than 10^{10} scFvs. Among all ScFvs isolated one of them, named ScFv6, showed the best perfomance.In order to clarify the mechanisms of neutralization, docking and energy minimization calculations of the antibody-CTX were also conducted. From these simulations, three single changes were chosen to be mutated. Mutants S30A and Y31F had aminoacid changes in CDR H1 while R103H in CDR H3.**Objectives:** To produce scFvs antibodies with improved affinity viewing a possible therapeutic alternative for envenoming. **Methods:** The first mutant, S30A, was obtained by site-directed mutagenesis, while the others were obtained from synthetic genes with codons optimized for bacteria expression. ScFv original and mutants were cloned into pET20b+ vector and the constructions were used to transform C43 bacteria. The production of scFvs was accomplished by induction with IPTG. The mutated proteins and the original scFv were all expressed in soluble form. Periplasmic fractions were isolated through osmotic shock and further purified by Ni(2+)-immobilized metal affinity chromatography and the purity of scFvs was analysed by SDS-PAGE. Circular dichroism was performed to analyse the secondary structure of scFvs. **Results and Discussion:** Circular dichroism revealed that scFvs had preserved secondary structure. Sequencing confirmed the desired mutations. Results show that all mutants presented similar expression levels. ScFvs will be now analysed regarding their affinity to CTX by surface plasmon resonance assay.

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1.20 Transcriptomic profiles of the venom gland from*Tityus serrulatus,Tityus bahiensis and Tityus obscurus scorpions*

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Introduction: *Tityus serrulatus* and *T. bahiensis* are scorpions widely distributed in Brazil with the exception of North region where *T. obscurus* occurs. Scorpion venom is a mixture of toxins, including neurotoxins as well as ions channels blockers, which are responsible for the symptoms of clinical manifestation. Other known components are antimicrobial peptides, bradykinin-potentiating peptides, anionic peptides, metalloproteinases and phospholipases. **Objectives:**Here we sequenced and analyzed the transcripts from the venom glands of these three species, aiming at identifying, annotating and comparing the venom expressed genes. **Methods**: Telsons were removed after being milked by electrical stimulation.ThemRNA extraction was carried out with oligodT magnetic beads. Sequencing was performed in a GS Junior 454 Sequencing System following the manufacturer protocols. Sequences were assembled with Newbler Software and RNA-seq quantification performed with CLC Genomics Workbench. Consensus sequences were subjected to a BLAST search and an Automatic annotation using Blast2Go. **Results and Discussion:** Annotation identified transcripts with high degree of similarity to known scorpions toxins and also to products involved in cellular process. Toxins represent 7%, 10% and 14% of gene expression in the *T. bahiensis,T. obscurus and T. serrulatus* glands, respectively. It was possible to identify potassium and sodium channel toxins, metalloproteinases, antimicrobial peptides, anionic peptides, and bradykinin-potentiating peptides. Regarding non-toxin transcripts, several sequences code for actin, tubulin, ATPases, splicing factor, transposases and mitochondrial proteins among other cellular proteins. We provide the first attempt to massively identify the components of these three species and one of the few transcriptomic efforts on the genus *Tityus*.

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1.21 Biochemical and enzymatic characterization of *Acanthoscurria gomesiana* Mello-Leitão, 1923 spider venom (Araneae: Theraphosidae)

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Introduction: The genus *Acanthoscurria*spidersfamily Theraphosidae (infraorder Mygalomorphae) are the biggest spiders of the world.These spiders are distributedworldwide mainly in tropical or semi-tropical areas.Their ecologicaldiversity is extensive, and includes dry or humid areas,savannas, deserts, rainforests or semi-temperate habitats. The contact between humans and spiders is frequent, mainly in breeding period, andit may cause accidents.The main clinical symptoms presented by patients bitten by *Acanthoscurria gomesiana* are local pain, erythema and edema.To date, there are no studies characterizing *A. gomesiana* spider venom. Few reports are dedicated mainly to investigate antimicrobial peptides andpolyamines present in the hemolymph of *A. gomesiana*. **Objectives:** The aim of this work was characterize biochemical and enzymatic propertiesof*A.gomesiana*spider venom. **Methods:** SDS-PAGE (12%)silver stained was employed to determine the electrophoretic profile of *A. gomesiana* venom (20 µg). In order to verify the enzymatic activities in *A. gomesiana* venom (100 µg), zymography was performed in a polyacrylamide electrophoresis gel (12%) using fibrinogen (0.5 mg/mL), casein (2 mg/mL), gelatin (2 mg/mL) and hyaluronic acid (170 µg/mL) as substrates. **Results and Discussion:**Theelectrophoretic profile of *A. gomesiana* venom presented of 23 components distributedin the range of12-250 kDa.Strongly stained bands were observed around 12 kDa, 17 kDa, 55 kDa and above 140 kDa. Moreover, densely stained bands were also present between 25-50 kDa. Gelatinolytic and caseinolytic activities were intensely detected in *A. gomesiana* spider venom, with bands located around 30 kDa and above 100 kDa. Furthermore, some spots were also observedin the region of 24 kDa in both substrates.Strong fibrinogenolytic activity was observed at 60 kDa region. No hyaluronidasic activity was detected in *A. gomesiana* venom. Our results indicated that *A. gomesiana* venom has enzymes that can act directly on extracellular matrix components, contributing to its degradation. Futures studies will be performing in order to characterize the toxic activity *in vivo* of *A. gomesiana* spider venom.

Supported by FAPESP

1.22 Evaluation of different patterns of muscle injury and regeneration induced by toxins isolated from Bothrops venoms

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Introduction: Envenomations by Bothrops snakes represent about 80% of accidents in Brazil and the tissue damage observed can results in dysfunction of the affected organ. The jararhagin (JAR) and bothropstoxin-I (BthTX-I) toxins, from *Bothrops jararaca* and *Bothrops jararacussu,* respectively are involved in the inflammation and tissue injury by distinct mechanisms. Macrophages and neutrophils, as elements of the immune system, are involved in the resolution of the injury and tissue repair. **Objectives:**It was studied the cellular influx and the presence of classical and alternative macrophages (M1 and M2) during muscle injury and regeneration induced by JAR and BthTX-I. **Methods:** Groups of mice received different doses of JAR or BthTX-I in the gastrocnemius muscle and after 4, 24, 48, 72, 96h and 7 or 28 days it was analyzed thecreatine kinase (CK) levels, Myeloperoxidase (MPO) enzyme activity, cellular influx by histological and flow cytometry assays and the mRNA expression of Miogenin, iNOS and arginase by RT-PCR. The control group of mice received PBS. **Results and Discussion:** Mice injected with BthTX-I 4h before produced the highest CK levels. In contrast, the higher MPO activity was detected in the JAR-group. The neutrophils are the first cells to migrate to toxin site of injection with a peak at 4h that was maintained until 72h of toxins injection. Macrophages were higher and firstly evidenced at 48h in JAR-group and lower at 72h after BthTX-I injection. These cells were still verified after 7 and 28 days of BthTX-I injection. Later expression of Miogenin, was detected in BthTX-I-group compared with JAR-group. In contrast, higher expression of iNOS and arginase, markers of M1 and M2 macrophages, was verified in JAR-group. The results suggest that the muscle regeneration in BthTX-I group occurs later when compared with the JAR group. This difference may be influenced by the intense inflammation and cellular migration observed in JAR-group.

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1.23 Effects of an anti-muscarinic component isolated from Micrurus lemniscatus venom on intracellular signaling by inositol phosphate and learning and memory in rats

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Introduction: Elapid venoms usually exhibit muscarinic cholinergic components.**Objectives:**This study investigated the effects of MTMlβ isolated from *M. lemniscatus* venom, on (1) the displacement of the hippocampal (Hpc) muscarinic antagonist [^{3}H]QNB, (2) the levels of inositol tri-phosphate, and (3) performance of rats in the Morris' water maze. **Methods:**Inexperiments of saturation, Hpc membranes were incubated with [^{3}H]QNB (0,05-8,0 nM) both in the absence and presence of atropine (1 μM) (30°C/1h).Scatchard analysis of specific binding yielded a dissociation constant (K_D)=0.88 ± 0,13 nM and binding capacity (B_{max}) = 1459.40 ± 235.26 fmol/mg of protein (n=5). Hpc membranes were also incubated with [^{3}H]QNB, both in the absence and presence of increasing concentrations of the MTMlβ and atropine (control) (30°C/1h).**Results andDiscussion:**TheproteinMTMlβ exhibits a N-terminal sequence determined by Edman degradation (NLYQFKNMIQCTNTRSCLDFADYGCYCGRGGSCT) and displays high similarity to proteins from Elapidae venoms already described.MTMlβ and atropine revealed one high affinity muscarinic binding site (respectively, pK_i = 7.38 ± 0.15, n=4 and pK_i = 8.96 ± 0.08, n=4) to [^{3}H]QNB in the hippocampus. The MTMlβ (10^{-7} M) reduced the accumulation of intracellular [^{3}H] - inositol phosphates content induced by carbachol (10^{-5} M). Rats subjected to training in a working memory version of the Morris' water maze task, with the platform in a different location every day and 4 trials per day, received, on the 8th day, a Hpc injection (0.25μg/μL) of either MTMlβ or phosphate buffer twenty minutes before training. Their performance did not differ significantly from that exhibited by the Control subjects. On the 9th day, however, when tested in the same task without any microinfusion, the subjects previously injected with MTMlβ exhibited significantly longer latencies and path lengths as compared to the Control subjects (P < 0.0004), and significant longer times spent within the day before critial quadrant (P < 0.0083). These results indicate that the disruption of performance observed on the 9th day may be related to a better retrieval of the information about the platform location acquired on the previous day, during the MTMlβ effect.Hpc histology did not reveal any neuronal injury. In conclusion, theMTMlβ exhibits affinity for mAChRs and reduces the total inositol phosphates, revealing a profile of muscarinic antagonist in the rat hippocampus.

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1.24 Effects of the *Tityus obscurus* scorpion venom in laboratory animals

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Introduction: A great number of studies about Brazilian scorpions are available. However few it is known about the venom of scorpions from the northern, mainly about Tityus obscurus venom that causes the greater number of accidents in Amazonia. **Objective**: We proposed to perform an inventory of the fauna and to study the maintenance in captivity of scorpions collected in Santarém and Belterra/PA to study the pharmacological effects of the venom in rats and mice. **Methods:** We obtained 498 specimens of 08 scorpion species belonging to the Buthidae and Chactidae families. The scorpions were bred in the Arthropods Vivarium of the Butantan Institute; the venom was obtained by electrical stimulation of the telson. The *T. obscurus* venom was administered intraperitoneally (IP) in rats to determine: LD_{50}, the behavioral effects, the occurrence and intensity of pulmonary edema, histopathologycal changes in hippocampal cells and lung tissue, the action on convulsions induced by drugs and on the general activity. The venom administered (IP) to mice was compared with the *T. serrulatus* venom. We evaluated LD_{50}, the behavioral effects and the nociceptive and edematogenic activity induced by increasing concentrations of the venoms. **Results and Discussion:** In relation to the collections, the *Buthidae* family and the *T. obsucurus* species were predominant. Concerning the effects of the *T. obscurus* venom in rats, LD_{50} was not obtained and the behavioral effects were more intense in higher doses. In the morphological analysis of the lungs, we observed the presence of hemorrhagic points in the parenchyma. However, the venom did not lead to pulmonary edema. In the locomotor activities, the animals evaluated one hour after the envenomation showed a decrease in the parameters. The *T. obscurus* venom did not induce changes on the occurrence and intensity of convulsions. In the results of the experiments with mice, the LD_{50} observed was 62.59 µg/20g. The effects observed with the *T. serrulatus* venom were more intense than those observed with the *T. obscurus* venom. Both venoms induced edematogenic and nociceptive activity in mice. The nociceptive activity of the *T. obscurus* venom is lower than the one induced by the *T. serrulatus* venom. The venom of the *T. obscurus* scorpion probably has different characteristics than the venom of other *Tityus* that present convulsant effects and lead to death, mainly due to cardiorespiratory changes under very lower doses. Only the edematogenic activity presented an equivalent response for both the venoms tested.

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1.25 Antiproliferative effects of Jararhagin toxin on human breast adenocarcinoma

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Introduction: The toxins from snake venom are a biological source of different peptides, enzymes and toxins pharmacologically active, with great therapeutic potential. Jararhagin is a toxin isolated from the venom of *Bothrops jararaca* that structural domains present important biological functions, showing great versatility and consequent medical importance. The effects of Jararhagin *in vitro* on MCF-7 human breast cancer cell, were investigated viability, morphological changes and distribution in cell cycle phases. **Objectives:** Evaluated effects of Jararhagin potential antiproliferative and pro-apoptotic in MCF-7, human breast adenocarcinoma cell. **Methods:** Tumor cell line was evaluated of viability by MTT assay, after treatment with different concentrations with the toxin. The LPO test was used to assess the formation of lipid peroxid radical. Cell cycle phases were assessed by flow cytometry. When exposed to higher concentrations cell lines were evaluated morphological changes in Confocal microscopy. **Results and Discussion:** IC50% was obtained with value of 20.3 μM showing a significant cytotoxic effect on tumor cell. Jararhagin treatment resulted in a significant lipid peroxid radical formation. The flow cytometric analysis showed changes of cells in different phases of the cell cycle and cell ploidy ratio. Confocal Microscopy was used to visualize the morphological changes of MCF-7 cells in monolayer and its formation as spheroids after treatment.

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1.26 Investigation of inorganic elements in *Lonomia obliqua* species from Brazil by EDXRF technique

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Introduction: The *Lonomia obliqua* (Lepidoptera: Saturniidae) has great medical importance because the human envenoming by larvae, especially in South and Southeast Brazil can produces mild cutaneous reaction as erythema, some edema and pain, and severe systemic reactions that include consumptive coagulopathy, intracerebral hemorrhage and acute renal failure, that can lead to death. Patients have the physiological coagulation parameters recovered after treatment with specific anti-serum produced at Instituto Butantan in horses against *L. obliqua* bristle extract. Since 2008, Brazil is the largest consumer of pesticides in the world according to ANVISA. The use of these products differs in various regions of the country in which intermingle traditional and intensive agricultural activities. Pesticides can be classified into chlorinated compounds or, pyrethrins, organophosphate, and carbamate. The exposure of pesticides to the environment can have a high or low environmental impact. **Objectives:** In this study we investigated the elemental composition of caterpillars with suspected pesticide contamination using X-ray Fluorescence (EDXRF) techniques. **Methods:** The biological materials came from States RS and SP to Butantan Institute. Two samples each State was investigated, the caterpillars were anesthetized, frozen with dry ice, macerated and lyophilized. For this investigation was utilized the X-ray Fluorescence analysis performed in an EDXRF Spectrometer SHIMADZU Co. model Rany 720 (50kV, 100 μA- variable, Rh target) and Si(Li) detector. **Results and Discussion:** The concentration values for Not Healthy caterpillars were determined for: (ugg^{-1}) Si (317±34), Fe (191±8), Zn (166±4), Cu (41±3), Ti (23±4), Mn (26±4), Rb (5.5±1.0), Br (6±1), Ni (6±2), Sr (1.5±1.0), ($gkg^{-1)}$K (33.5±0.2), P (5.2±0.6), S (5.7±0.3), Cl (5.1±0.2), Mg (3.9±0.4), Ca (2.3± 0.3).The concentration values for Healthy for: ($ugg^{-1)}$ Si (49 ±15), Fe (55.5±4.5), Zn (64.5±3.0), Cu (35±4), Ti (9±4), Mn (59±4), Rb (2±1), Br (5±1), Ni (5±2), Sr (<1 ^{1}), ($gkg^{-1)}$K (21.5±0.2), P (5.19±0.06), S (5.8±0.3), Cl (2.5±0.2), Mg (1.6±0.2), Ca (0.40± 0.03). These preliminary data suggest that there were differences between healthy and unhealthy caterpillars, particularly to P and Cl. Considering that these elements are part of the composition of most pesticides, the concentration increase (P and Cl) can be affect the development of these animals as well as the quality and yield of serum. Related to the EDXRF technique, it is fast and not destructive procedure and also permit to perform analyses using small quantities of samples.

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1.27 Comparison of phylogeny, venom composition and neutralization by antivenoms in snakes of *Bothrops* complex

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Introduction: Frequently the venom composition has been considered as a reflex of snake phylogeny, with implications for the production of antivenoms and treatment of envenoming. **Objectives:** The aim of this study was to analyze the composition of venoms from 6 species of snakes from *Bothrops* complex and their correlation with snake phylogeny and reactivity with *Bothrops* Antivenom (SAB). **Methods:** Venoms from *Bothropoides jararaca*, *Bothropoides neuwiedi*, *Bothrops atrox*, *Bothrops jararacussu*, *Rhinocerophis cotiara* and *Rhinocerophis alternatus* were analyzed by SDS-PAGE and reverse phase chromatography on HPLC using C18 column. The reactivity of SAB with venoms and their fractions was performed by ELISA and western blot, followed by assessment of neutralization of lethality and hemorrhagic activity of the venoms of *B. jararaca* (present in immunization pool of SAB) and *B. atrox* (absent from the pool). **Results and Discussion:** The venoms showed distinct electrophoretic and chromatographic profiles, without apparent correlation with the snake phylogeny. Only venoms of the genus *Rhinocerophis (R.alternatus* and *R.cotiara*) showed relatively similar profiles. SAB recognized the venoms of different species with the same antibody titer of 640,000. Most of the fractions eluted from C18 column were recognized by SAB, especially those corresponding to snake venom metalloproteinases (SVMP) of class P-III. By SDS-PAGE the venom of *B. jararacussu* presented more distinct electrophoretic profile with predominance of phospholipase A_2, while in other venoms, the SVMPs were the predominant components. Two µL of antivenom neutralized more than 50% of the hemorrhage induced by 10 µg of either venom (*B. jararaca* or *B. atrox*); this proportion was also sufficient to protect more than 50% of the mice from the challenge with 3 LD_{50} of *B. jararaca* venom (105 µg), although for *B. atrox* (225 µg) the same effect was only obtained when doubling antivenom amount. Thus, we conclude that SAB react similarly with the same family of toxins from distinct venoms regardless of the phylogeny of the snake or the presence of venom in the immunization pool employed in SAB production. This suggests that a *Bothrops* antivenom with greater efficiency and broad spectrum can be developed, if one considers the reactivity of antivenom to the different classes of toxins and not just the phylogeny of snakes.

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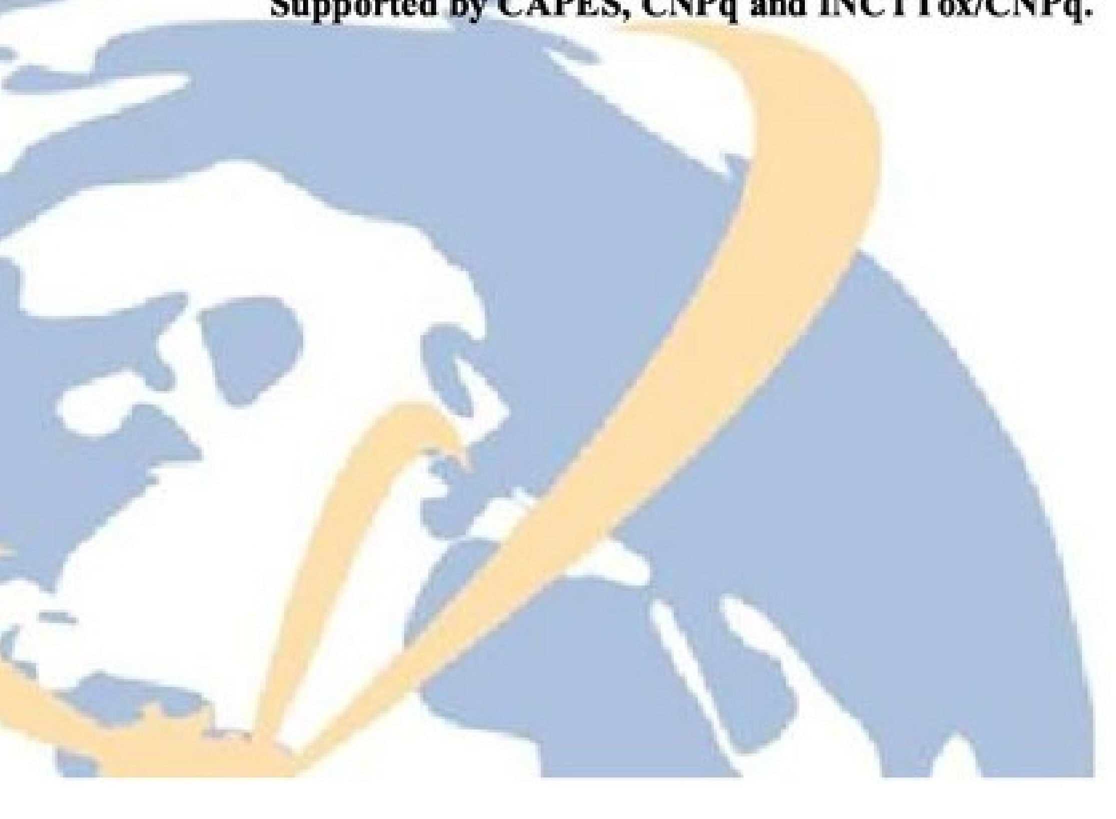

1.28 Proteomic analyzes of *Bothrops atrox* venom from FLONA-Tapajós reveal the presence of unusual components

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Introduction: *Bothrops atrox* is responsible for most snake bites reported in the Brazilian Amazon and biochemical and epidemiological data suggest that the clinical manifestations of this envenoming may vary in different regions. The intraspecific variation of snake venoms represents a well-documented phenomenon, particularly evident among the species that have a wide distribution, like *B. atrox*. Venom variability occurs due to a number of factors, including the geographical distribution. **Objectives:** To study the variability in the venom composition of *Bothrox atrox* snakes captured at Floresta Nacional do Tapajós (FLONA-Tapajós).**Methods:** Venoms of 8 adult specimens, males and females, were pooled and 2 mg of the pool were dissolved in 250 μL of trifluoroacetic acid (TFA) 0.1% and submitted to reverse-phase chromatography on HPLC using a C18 column. Twenty one fractions were collected and subjected to tryptic digestion followed by analysis by nanoESI-LTQ/Orbitrap.**Results and Discussion:** The results revealed that the proteome of *B. atrox* from FLONA-Tapajós has a high content of snake venom metalloproteases (SVMP), especially from class P-III. Phospholipase A2, serino protease, L-amino acid oxidase, cysteine-rich secretory protein and C-type lectin were also detected in accordance with previously described data. However, our analysis also indicates the presence of components not usually described in proteomic data already published for this species, such as: nerve growth factor, vascular endothelial growth factor, ecto-5'-nucleotidase, hyaluronidase, glutaminyl cyclase and phospholipase B. In conclusion, this work demonstrated the presence of unusual proteins in proteomes of *B. atrox* and their presence suggests that the pre-fractionation of venom may have facilitated identification of less abundant components. Furthermore, the identification of unusual components in the venoms of *B. atrox* from FLONA-Tapajós can also suggest possible differences in clinical manifestations resulting from envenoming by these snakes.

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1.29 Inflammatory gene expression in the lung of mice geneticallyselected for acute inflammatory response and treated with *Tityus serrulatus* venom

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Introduction:*Tityus serrulatus* is the main cause of scorpion accidents in Brazil. Cardiovascular failure complicated by pulmonary edema is the main cause of death after severe envenomation. *T. serrulatus* venom (TsV) induces a systemic inflammatory response with the release of inflammatory mediators both in patients and animal models. Genetic factors may influence the severity of symptoms reported by patients. We have previously shown lung alterations with the presence of inflammatory infiltrating cells and edema in mice genetically selected for high acute inflammatory response (AIRmax).**Objectives:**The aim of this study was to evaluate inflammatory gene expression in the lung of AIRmax and AIRmin TsV treated mice. **Methods:** AIRmax and AIRmin were inoculated with 0.75 µg/g bw of TsV and after different time points, the animals were euthanized (n = 3 per period). Lungs were flushed with saline, infused with RNA later® and then stored at -20ºC. Total RNA was isolated using the RNAspin isolation kit (GE Healthcare, UK). RNA integrity was checked with Agilent Bioanalyzer and 500 ng high-quality samples were reverse transcribed using Superscript III™ reverse transcriptase (Invitrogen, USA), oligo-dT primer and double-stranded complementary DNA (cDNA). Quantitative real-time PCR was performed with gene-specific primers for *Il-6, Il-1b, Tnfa* and *Cxcl1* (KC chemokine) using the Fast SYBR® Green kit and run in a StepOnePlusTM thermocycler (Applied Biosystems, USA). Relative quantification of target genes was analyzed with the StepOne software using the Cycle threshold (Ct) method, normalized for *B2m* and *Rps29*endogenous genes and control groups as the calibrators. **Results and Discussion:** Kinetics of gene expression in the lungs showed a significant increase ($p < 0,001$) of *Il1b*, 4 hours after venom inoculation in AIRmax TsV-treated mice, compared to AIRmin ($p < 0,01$). It was also observed an increase of *Il-1b*, 30 minutes after venom inoculation compared to controls in both lines. Moreover, there was an increase of *Il6* during all periods in AIRmin mice, although AIRmax presented higher basal expression. There were no significant differences in *Tnf* expression between the two lines and to respective control groups. Our results suggest that *T. serrulatus* venom is able to induce differentially inflammatory gene expression in the lung of AIRmax and AIRmin mice, suggesting the importance of genes selected for acute inflammation in the response to animal venoms.

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1.30 Proteomic and peptidomic characterization of venoms from the spider *Acanthoscurria gomesiana*

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Introduction: The order of spiders (Araneae) is considered one of the most diverse in number of species, and also one of the most successful among poisonous animals on Earth. One of the key factors to the success of spiders is the production of a highly toxic venom from their glands, used to subdue prey and for protection against potential predators. These components are primarily proteins and peptides which act mainly as neurotoxins, cytotoxins, and as ion channel modulators. However, despite several advances, the number of proteins and peptides isolated and characterized from spider venoms is estimated to be only 0.01% of all possible sequences. **Objectives:** Considering the biological potential of spider venom toxins and the lack of studies from Brazilian spiders, the objective of this work is to characterize the protein and peptide composition of the venom from *Acanthoscurria gomesiana* by mass spectrometry, prospect molecules with biological activity and identify sex-based differences. **Methods:** The venoms were obtained from adult male and female specimens separately. Venoms were fractionated by RP-HPLC and the fractions were used in antimicrobial and platelet aggregation assays. The fractions were submitted to SDS-PAGE, mass spectrometric analysis and database search. **Results and Discussion:** The venoms of male and female specimens of *Acanthoscurria gomesiana* present differences in composition. In males, the average protein concentration was 83.53 mg/mL, while in females it was 62.04 mg/mL. In addition, RP-HPLC and SDS-PAGE analysis indicate differential expression of peptides and proteins. Some fractions presented antimicrobial activity and protein bands demonstrated peptide similarity with a neurotoxin theraphotoxin from two species of arachnids, *Acanthoscurria natalensis*and *Lasiodora parahybana.*The analysis of de novo sequencing from same fractions showed a large number of sequences with high probability of existence in fractions of male and female venon, reflecting the importance and need of continuing with this type of work.

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1.31 Effects of *Scolopendra viridicornis* centipede venom on the mast cell and histamine release *in vitro* and *in vivo*

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Introduction: Centipedes are regarded as the oldest and largest terrestrial arthropods belonging to the class Chilopoda of the subphylum Myriapoda. Although centipedes have long been known to be venomous, their venoms remain largely unexplored. Concerning the venom from the Brazilian centipede *Scolopendra viridicornis*, specie commonly found in many regions of the country, bites is generally mild, and human victims usually manifest burning pain, paresthesia and edema. The proposed treatment is mainly the administration of analgesics, local anesthetics, antihistaminic drugs, among others. Previous report has shown that *Scolopendra viridicornis* (*Sv*) venom exhibited phospholipase A_2, fibrinogenolytic, caseinolytic and gelatinolytic activities. Besides, strong hyaluronidase and intense direct hemolytic activities were also observed. In relation to studies *in vivo*, venom caused nociception, myotoxicity and a rapid and persistent edematogenic activity followed by leukocyte influx in mice. **Objectives:** The aim of this work was to verify the involvement of mast cells and histamine in edema induced by *Sv* venom. **Methods:** Groups of Swiss mice were pretreated with cromoglycate (inhibitor of mast cell degranulation) or the histamine-receptor antagonists promethazine (H_1R antagonist), cimetidine (H_2R antagonist), or thioperamide (H_3/H_4R antagonist), and injected i.pl. with *Sv* venom (15 µg/paw) to evaluate edema forming. Edema was measured by plethysmometer (15 min, 30 min, 1, 4, 6, 24 and 48 h). **Results and Discussion:** The maximum peak was observed at 15 min after *Sv* venom injection, returning to baseline within 48 h. Cromoglycate significantly reduced paw edema (40-90%) induced by *Sv* venom in all periods evaluated. The pretreatment of mice with promethazine (all periods varying between 50-100%) and thioperamide (4 h, 30% and 24 h, 32%) decreased paw edema caused by *Sv* venom. On the other hand, cimetidine did not reduce edema in any of the evaluated time periods. In addition, *Sv* venom (7.5; 15; 30 and 60 µg/well) induced mast cell degranulation in both PT-18 (mouse mast cell line) and RBL-2H3 (rat basophilic leukemia) cell lineages, with no effect on cell viability. In conclusion, mast cells and histamine release are involved in local reaction induced by *Sv* venom; however, other mechanisms may be involved in the inflammatory process caused by *Sv* venom.

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1.32 Asynchrony in the synthesis of toxins by primary culture of snake venom gland secretory cells

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Introduction: The main venom gland of Viperidae snakes has an important characteristic that is the presence of central lumen, where the venom produced is stored. When the venom is lost from the gland, the secretory cells are activated and venom production starts. The venom production cycle is long and lasts around 30-50 days. However, our research group showed that toxins are present in the gland in quiescent stage and the synthesis of new toxins are asynchronized. Besides, we showed that secretory cells from venom gland in quiescent stage is able to produce and secrete venom to the medium. Therefore, we can consider quiescent stage as a basal stage.**Objectives:**Thus the aim of this study is to identify and to compare the toxins produced and secreted by the secretory cells of the venom gland in quiescent stageduring culture time.**Methods:** The primary culture of secretory cells was done (n=3), and the culture media were collected every three days until the twelfth dayfrom 0 to 3 days of culture (3 D), from 3 to 6 days of culture (6 D), from 6 to 9 days of culture (9 D) and from 9 to 12 days of culture (12 D). The albumin was removed from the media. The culture media collected from different times were subjected to two-dimensional gel electrophoresis (2-DE). The gels were analyzed with software ImageMaster 2D Platinum. The culture medium was used as a negative control. In order to recognize the venom in the media, Western blotting assay was done using antibody against *Bothrops jararaca* venom.**Results and Discussion:** The analysis of the 2-DE images of culture media showed the appearance of new spots in 3 D culturemedium (ranging from pI 4.6 to 4.97, 59 kDa; ranging from pI 3.7 to 3.92, 60 kDa; pI 5.4, 48 kDa and pI 5.7, 38 kDa), in6 D culturemedium (pI 5.75, 45 kDa; ranging from pI 3.2 to 3.71, 73 kDa; pI 3.57, 67 kDa; pI 5.55, 41 kDa and pI 6.3, 40 kDa), in 9 D culturemedium (pI 3.03, 65 kDa, pI 5.9, 21 kDa; pI 5.6, 40 kDa; pI 6.05, 39 kDa; pI 5.24, 91 kDa and pI 5.35, 107 kDa; pI 8.2, 76 kDa) and in 12 D culturemedium (pI 5.7, 40 kDa; pI 5.0, 73 kDa; pI 3.5, 71 kDa and pI 8.1, 77 KDa). Western blotting assay revealed the presence of proteins of the venom in the culture media with MW ranging from 18 kDa to 78 kDa and pI from 3.0 to 8.0. In this study we detected the presence of new spots during culture time, suggesting that the synthesis of venom proteins *in vitro* is asynchronized as occurs *in vivo*. The identification of these proteins allows us to understand the dynamic of toxins production in culture and allow us to get news insights about the mechanisms involved in venom gland activation and production of venom, contributing to studies about snake toxins and their diversity.

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1.33 Expanding the known substrate degradome of snake venom metalloproteinases by mass spectrometric analysis using PICS and TAILS

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Introduction: Snake venom metalloproteinases (SVMPs) play important roles in the pathological effects of viperid venoms including local tissue damage, hemorrhage and coagulopathy. Hemorrhagic Factor 3 (HF3), a metalloproteinase isolated from the venom of the snake *Bothrops jararaca*, induces severe local hemorrhage by synergistic effects upon plasma, extracellular matrix and platelets. Previous proteomic studies have shown that HF3 targets important components of extracellular matrix, such as collagens and proteoglycans, and some plasma proteins. However the full substrate repertoire of this metalloproteinase is unknown. **Objective:**the aim of this study was to determine the specificity of HF3 as well as its substrate repertoire (degradome). **Methods:** using PICS (proteomic identification of cleavage sites) a proteome-derived peptide library was used as substrate for identifying protease cleavage sites by liquid chromatography coupled to high-resolution mass spectrometry (LC-MS/MS) and bioinformatic analysis. In addition, the degradome of HF3 was evaluated using a recent described proteomics approach named TAILS (Terminal Amine Isotopic Labeling of Substrates) in which neo-N-termini (derived from cleavage of intact proteins with a proteinase of interest) are enriched and analyzed by LC-MS/MS. **Results and Discussion:** We determined over 2000 cleavage sites and analyzed sequence preferences within the full P6 to P6′ range. Hydrophobic residues were preferentially found at the P1' site with leucine, isoleucine and phenylalanine accounting for 40%, 11% and 7%, respectively, of all residues at this position. In addition, terminal amine isotopic labeling of substrates (TAILS) derived from the incubation of HF3 with mouse embryonic fibroblasts secretome followed by LC-MS/MS analysis resulted in the identificationof more than 500 cleavage sites in native proteins. Various novel substrates were detected for HF3 after peptide isotopic quantification and bioinformatic analysis including the cysteine proteinase inhibitor,cystatin-C, and the apoptosis inducer,galectin-1. Taken together, these results greatly expand the known substrate degradome of HF3, and reveals new targets which may serve as basis to better elucidate the complex pathophysiology of viperid snake envenomation.

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2. Biochemistry

2.01 Preliminary proteomic study of saliva from patients with Sjögren's Syndrome

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Introduction: Xerostomia is the subjective sensation of dry mouth, consequential without or with reduction or interruption of salivary gland function. It is a common and primary symptom during clinical practice, and it belongs to the complex exocrinopathy that affects the salivary glands in Sjögren's syndrome (SS). These patients often exhibit dryness on the lips, tongue and pharynx and the resulting soreness and burning of mucous that hinder speech, chewing, swallowing and digesting food. The pathogenesis of xerostomia in exocrinopathy is complex and only partially understood. **Objectives:** To perform a preliminary qualitative proteomeof the saliva from patients with Sjögren's Syndrome. **Methods**: The unstimulated saliva was obtained from 12 patients with Sjögren's Syndrome, and 6healthy volunteers. The total proteins from salivary were extracted, measured using the BCA Protein AssayReagent. The proteins were precipitated with acetone 1:4 (v/v), digested with trypsin, reduced and alkylated, before their analysesbyliquid chromatography/mass spectrometry (MS/MS) ion traptime-of-flight (LC/MS-ESI-IT-TOF). **Results and Discussion:** The resultobtained from the comparison of salivary samples indicatedchanges of Ig alpha-1 chain C region and NADH-ubiquinone oxidoreductase chain 5. Bioinformatics-assisted pathway analysis of the proteins revealed that protein present on salivary was significantly affected. This preliminary study suggests that clinical changes of the salivary glands might be reflected in patients with SS. The qualitative analyses of the salivary proteomes from these patients are quite important for clarify the pathogenesis mechanisms of xerostomia in SS and also for the search of a proper diagnosis and treatment to these patients.

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2.02 Characterization of cryptides obtained through hydrolysis of myoglobin by serine proteases from the venom of *Bothrops jararaca*

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Introduction: Snake venoms are complex mixtures, constituted by enzymes such as phospholipases A_2, metalloproteases and serine proteases that act on the victim's tissues and proteins. As a result of their direct actions on tissue proteins, these proteases could generate peptides with specific actions in cells or other mechanisms. Recent studies have shown that a new class of bioactive peptides, cryptides (CRYP). **Objectives:** Characterization of cryptides generated through the action of serine proteases from the venom of *Bothrops jararaca* on myoglobin. **Methods:** The serine proteases (VSP) were separated from the venom using a benzamidine sepharose 4 FF. Myoglobin (Myo) was incubated with either VSP, commercial trypsin (CT) or the whole *Botrhops jararaca* venom (WVBj) for different times. The CRYP were isolated by HPLC (C18-RP column), and evaluated on HUVEC cells cultures by MTT assay. Both CT and VSP generated two active peptides that were sequencedand synthesized (C1 and C2). To evaluated which phase of the cell cycle these CRYP showed activity, the cells were treated with 0.5 and 5 μM C1, C2 or untreated and the cell cycle was analyzed by FACS. Also was performed kinetic analysis of cell growth using BioStation for 72 h. To determine the action of these CRYP was performed angiogenesis assay, cells were plated on Matrigel and were treated with C1 or C2 at concentrations 0.05; 0.5 and 5 μM. **Results and Discussion:** Serine fraction has been possible to verify that some of the isolates are already CRYP generated from ½ h period, remaining 36 h. However, when the Myo was incubated with WVBj was possible to identify CRYP but after 3 h was almost undetectable. The MTT assay showed that cells treated 8 μM of C1 induced 29.5% cell proliferation and 6 μM of C2 induced 27.41%. C1 and C2 increased the proportion of cells in the S phase C1 showed 15.33% at 0.5 μM and 9.63% at 5 μM and C2 increase 8.33 % at 0.5 μM and 6.73 % at 5 μM. The kinetics of growth HUVEC´s, showed the cells treated with 0.05; 0.5 and 5 μM of C1 showed proliferative activity of 71% and 17% at 0.05 and 0.5 μM and cells treated with C2 showed cell proliferation of 35.6%, 18.2% and 34.7% respectively. The angiogenesis assay showed with C1 increased 16.13 % and 41.93 % at 0.05 and 0.5 μM, and C2 increased 7.26% at 0.05 μM. These results suggest that these cryptides are generated in the body after the bite of *B. jararaca*, suggesting new aspects in the process of poisoning and increasing the relevance of these cryptides.

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2.03Bioavailability studies of Amblyomin-X

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Introduction: Amblyomin-X, a 12 kDa recombinant protein, expressed in a prokaryote system (*E. coli*), is characterized as Kunitz-type inhibitor, acting on the FXa of blood coagulation. Moreover, the protein causes tumor remission in mouse implanted with melanoma cells, but apparently do not affect the normal cells. This protein is under preclinical phase, and bioavailability is one required parameter for this study. **Objectives:** In this study, was evaluated the bioavailability of Amblyomin-X in Swiss mice plasma.**Methods:** In the experiment, Amblyomin-X (20 mg/kg), diluted in sterile saline or control (sterile saline), was injected intravenous in right eye plexus mice and blood samples were collected (left plexus eye) at different times (0, 5, 15, 30, 45 and 60 minutes). The samples were centrifuged and the plasma was analyzed by SDS-PAGE 10% in reducing conditions, and Amblyomin-X corresponding bands were removed and digested for mass spectrometry analyzes, using a LC-ESI-IT-ToF (MS/MS). **Results and Discussion:** These findings indicate that Amblyomin-X was present in the plasma at 15, 30 45 and 60 minutes. More experiments should be performed in order to analyze the plasma more than 1 hour after injection, 120 min, 24, 48 and 72 hours or until the time that Amblyomin-X cannot be found. In conclusion, Amblyomin-X can become as a promising drug to prevent thrombosis and intravascular coagulation in cancer patients.

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2.04 Initial biochemical and structural characterization of the major skin secretion components of the anuran *Dermatonotus muelleri*

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Introduction: The skin of amphibians is replenished of interesting molecules to be studied, such as alkaloids, steroids, peptides and proteins. Frogs have few resources against predators, leading them to produce substances capable of avoiding and/or repelling predators, as well as protect against microorganism infection. *Dermatonotus muelleri* is known as "glue toad" and it is the only species of its genus. **Objective:** Since this species is poorly studied, this work aimed to study the skin secretion of *D. muelleri* aimed to obtain the biochemical profile and characterize the classes of molecules present in this anuran. **Methods:***D. muelleri* were submerged in a recipient containing deionized water and compressed manually. After skin secretion releases, the solutions were lyophilized, pooled, resuspended in appropriated buffer and analyzed by biochemical techniques. The skin secretion solution was analyzed by reverse-phase high-perfomance liquid chromatography coupled with photo diodes array detectors (RP-HPLC-PDA) using a C_{18} column (250x 4,6 mm) and Milli-Q water (A) and methanol (B) as mobile phase. To determinate the molecular mass of the components, fractions collected were analyzed by liquid chromatography mass spectrometry and mass spectrometry direct (LC-MS and MS) using two different ionization source - electrospray or atmospheric-pressure chemical ionization (ESI or APCI) - and both positive and negative ionization mode. **Results and Discussion:** The chromatogram profile shows several peaks present in skin secretion of *D. muelleri*. However, we decide primarily collect and study only a single peak noted at 320 nm. The sample was analyzed by mass spectrometry, though the molecules have not ionized well, which led us to test other methodologies. The most appropriate methodology uses the APCI source in negative mode. The preliminary analyses by mass spectrometry revealed the presence of low molecular mass molecules; however, complementary analyses are still necessary and are currently being carried out. The 320 nm absorbance and the low m/z detected values indicate that this molecule may be a tryptophan derivative; nevertheless, the structural characterization of this secretion has proved particularly challenging in terms of structural elucidation by mass spectrometry.

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2.05 Lacrain: first antimicrobial peptide from*Scolopendra viridicornis*.

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Introduction: Arthropods constitute one of the oldest groups of organisms, and also show a wide distribution in different ecosystems and habitats. This fact leads to the question about which factors allowed such an ecological success in these organisms. Knowing that most of the environments where they are found show a high presence of microorganisms and pathogens, it can be affirmed that part of the success of arthropods in colonizing these environments are their immune systems. One of the main components of the defense mechanism in vertebrates and invertebrates are the peptides with immune functions, which control the invasion of the different pathogens. The defensive role of a variety of antibiotic peptides in multi cellular organisms is increasingly recognized, but the characterization of these peptides had not begun until recent times. For these reasons, it is important to study this subject, not only to understand the success of these invertebrates and their defense mechanisms, but also to find alternatives to fight infectious diseases that affect humans. This is why the purification and characterization of these peptides and the knowledge of the function of their immune systems becomes even more interesting. **Objectives:**The aim of this study was the separation, analysis and characterization of bioactive compounds in the extract of the body of the Brazilian myriapod *Scolopendra viridicornis*. **Methods:**The bodies of the animals were first subjected to maceration and acid extraction and then fractionated in two steps. First, using C18 Sep Pak column cartridge, the hydrophilic and hydrophobic fraction was separated. For the second purification step, the hydrophobic fraction was purified by high performance liquid chromatography using a semi-preparative and analytical C18 Jupiter columns with a linear gradient of ACN in TFA 0.05%. The antimicrobial activity was determined by liquid growth inhibition assay. For the characterization we use mass spectrometry (MALDI-ToF) and the primary structure was obtained by De Novo sequencing using a mass spectrometry (Q-TOF). After that the molecule was synthetized and had his antimicrobial activity tested. **Results and Discussion:** In the total extract of the *S. viridicornis*'s body, different fractions with anti-microbial activity were observed. A fraction active against the bacteria Gram negative *E. coli* present a molecular weight of 925.5 Da peptide, and a primary structure of 8 amino acid residues (RYPAVGYT). This molecule was named *Lacrain.* After synthetized, the activity against bacteria *E. coli* and *Salmonella serovars* was confirmed. More analysis with the synthetic are necessary to determine the real potential of this molecule.

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2.06 Bioactive molecules of cobweb spider *Nephilengys cruentata* (Araneae, Nephilidae)

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Introduction: Spider webs are made from protein strands produced by the animal, which can synthesize many kinds of silk with different functions and mechanical features. Among other purposes, the webs can be used for ootheca construction, transport of sperm, shelter construction as well as prey-capture devices. Beyond the mechanical properties, it has been seen that the webs of some species of spiders have different lipid and protein compounds, which may be involved in many roles such as pre-digestion and paralysis of preys, chemical protection against predation and microorganisms and protection against degradation by fungi. These molecules, that are produced and deposited on the webs by spiders, can be the first step of the search for new drugs such as insecticides, repellents and antimicrobials agents. **Objectives:** In order to find new molecular compounds with bioactive potential presents in webs of *Nephilengys cruentata*, the aim of the project is the isolation and chemical characterization of these molecules, extracted from spider silk. **Methods:** In this stage of the study, the molecules were obtained from silk threads that were collected directly from specimens of Instituto Butantan and Universidade de São Paulo, SP, Brazil. The extraction was made by washing of silk with acetonitrile 50% and this solution was purified by RP-HPLC. After that, the isolated compounds were analyzed with mass spectrometer Surveyor MSQ Plus. The tests of bioactivity with the isolated samples were performed by growth inhibition assays with liquid antimicrobials. The molecules were tested against Gram negative bacterias. The posterior structural characterization of the bioactive molecules involved analyses of mass by MALDI-TOF-MS and LC-MS. **Results and Discussion:** The test of activity against *Micrococcus luteus* A270 indicated that one of the samples, purified by RP-HPLC, showed activity in low concentration. Posteriorly, the molecules will be tested against Gram positives bacterias as well filamentous and mold fungus. After mass analysis, a peptide was identified and its aminoacids sequence will be synthesized for new tests. With analysis in database, via Mascot, we verified that it can be possible that this sequence is a fragment of a type of spidroin, structural protein that make up the bulk of spider silk fibers. Due to the study is restringed to silk collected directly of the animals, these compounds are probably originated by silk glands. In another stage of the study we expect to analyze webs that are builded in nature with the view to compare the results that could indicate another origin, such as venom and gastric liquid, of newer compounds in orbwebs.

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2.07 Possible synergic effect of fallaxin and alkaloids from the skin secretion of *Leptodactylus labyrinthicus* in the rate of rabies virus mammal cells infection

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Introduction: Rabies is a zoonosis distributed worldwide, responsible for approximately 55 thousands deaths per year. It is characterized as alethalprogressiveacuteencephalitis, caused by a virus (*Rabies virus*; RABV). As in other therapeutic classes, molecules with antiviral effects may be present in differentorganisms, whose toxins, poisons and secretions have been studiedas sources of new bioactive substances. In the amphibian's case, their skin and skin secretions contain a great diversity and variety of bioactive molecules as alkaloids, steroids, proteins, peptides, among others. It is well known that antimicrobial peptides like fallaxin and pentadactylin were isolated from the skin secretion of *Leptodactylus labyrinthicus*. **Objectives:**This study evaluated whether molecules obtained from the skin secretion of *L. labyrinthicus* could interfere on the RABV infection. **Methods:** The skin secretion of *L.labyrinthicus* was mechanically obtained by compressing the animal submerged in water. This secretion solution was lyophilized, ressuspended in appropriated buffers and filtered in 10 KDa cut-off membranes. The filtered content was fractionated by Reversed Phase High Performance Liquid Chromatography(RP-HPLC), using a C18 monolithic column and fractions were assayed for cytotoxic and antiviral activity - in BHK-21 cells. After verifying that no fraction was cytotoxic, the evaluation of the possible effects of these fractions on RABV penetration, *PV* strain, was assessed through tests based on Rapid Fluorescent Focus Inhibition Test (RFFIT). The fraction able to reduce cells infection were identified and characterized by mass spectrometry.**Results and Discussion:**One fraction was able to reduce RABV infection and fallaxin, (identified and characterizes by means of ESI-MS^2),besides low molecular mass molecules, were present in this fraction. Synthetic fallaxin was obtained by peptide solid phases synthesis, but the level of RABV infection inhibition was diminished regarding the peptide present in the natural fraction, suggesting the a possible synergism between fallaxin and one of the low molecular mass molecules present in lower levels in the fraction. Complementary virological tests will be performed using synthetic fallaxin in association with purified bufotenin, an alkaloid with known antiviral action obtained from *Rhinella jimi*.

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2.08 Proteomics evaluation of FGF2 stimulus on adrenocortical carcinoma cell line through MudPIT technology and label free quantification

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Introduction: The fibroblastic growth factor 2 (FGF2) is associated with proliferation and carcinogenesis but anti-proliferative and tumor suppressive functions are observed in different cellular contexts. In Y1 murine adrenocortical carcinoma cell line, the FGF2 promotes G0 → G1 transition but delays S-phase and permanently block cells in G2/M. **Objectives:** To better understand the molecular mechanism unusually induced by FGF2 we performed mass spectrometry based quantitative proteomics focusing on the initial times of FGF-2 treatment. **Methods:** Y1 cells growing in DMEM medium were stimulated with FBS and FGF2 (10ng/ml) or FBS-only by 0, 3 and 5h. Total protein from all timepoints was extracted and enzymatic digested. Peptides were loaded on a MudPIT column and analyzed by LTQ-XL mass spectrometry on a fully automated 12-steps run. The data were processed by SEQUEST algorithm against a NCBI database of *Mus musculus* and filtered/compared using DTASelect and CONTRAST. Distributed normalized spectral abundance factor (dNSAF) were calculated using the algorithm NSAF7 and differentially expressed proteins with $p<0.05$ were obtained using PLGEM. The resulting data were analyzed by DAVID. **Results and Discussion:** More than 2900 proteins were identified/quantified by label free quantitative proteomics based on spectral counting and 250 of them were found differentially expressed ($p<0.05$, PLGEM) in FGF-2 treated samples. The resulting data were analyzed by functional clustering analysis of DAVID indicating that categories such as "DNA replication", "DNA metabolic process", "Chromatin assembly" ,"Nucleosome", "Splicing" and "Transcription" are differentially expressed after FGF-2 addition ($p<0.05$). Proteins such as GINS complex subunit 2 and replication factor C that are directly involved with DNA replication is downregulated mainly after 3h of FGF-2 treatment. Histone acetyltransferase, histone methyltransferase, polycomb protein Suz12 and arginine methyltransferase, among others are examples of chromatin remodeling factors that are differentially expressed after FGF2. We are currently validating the expression of 20 selected proteins associated with the above mentioned categories by western blotting and immunofluorescence. Taken together, DNA replication and chromatin remodeling factors as well as splicing machineries are differentially regulated by FGF2 suggesting that the signaling cascade of this growth factor might stimulate unknown chromatin components that, in turn, may play a role at the cell cycle blockage induced by FGF-2 in Y1 cells.

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2.09 Proteomic analysis of ontogenetic changes in juvenile and adult *Bothrops jararaca* liver

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Introduction: The liver is responsible for a wide range of critical functions essential to life. Because of its important roles performed in health and disease, characterization of the liver proteome is an essential step in fully elucidating its function. In addition to its role in metabolism, the liver synthesizes most of the proteins found in plasma. Besides, it is well known that the proteome is submitted to constant changes during development. The *Bothrops jararaca* is the main agent causing snake bites in Brazil. It is known that these animals are resistant to accidental envenomation, and that their plasma may play in this protection role. So, as the majority of plasma proteins are produced in the liver, it would be interesting to study their liver proteome. **Objectives**: The aim of this work is to analyze and compare the liver proteome during the development stages of juvenile and adult *B. jararaca*. **Methods:** Livers of juvenile and adult *B. jararaca* were collected and proteins were isolated according to TRIzol Reagent instructions (Invitrogen). IEF of liver proteins was undertaken using pre cast Immobiline DryStrip gels pH 3-10 gradient, followed by SDS-PAGE using 10% resolving gels, which were analyzed using ImageMaster Platinum 7.0 software (GE Healthcare). The spots were quantified using the % of spot volume criterion, which is automatically calculated by the ImageMaster software. **Results and Discussion:** 2D SDS-PAGE analysis revealed 464 matches among the two groups analyzed, with only 138 spots showing quantitative variation, in which 69 and 53 are exclusive for juvenile and adults, respectively. Besides, 7 and 8 are increased in juvenile and adults, respectively. The results showed that there are some differences in liver protein composition between juvenile and adult *B. jararaca* snakes. The perspectives of this work are to identify these proteins by mass spectrometry (MALDI-TOF).

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2.10 Lipase Analysis On Transcriptome and Proteome From Three Arachinida Species

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Introduction: Lipase superfamily is composed of a series of distinct proteins with several functions. Some Arachnida species present lipase (EC 3.1.1.3) activity in their digestive tract. However, little is known about which members of lipase superfamily are involved on Arachnida lipid digestion and their expression in distinct physiological conditions. **Objectives:** The aims of this work were: identify and classify digestive lipases in Arachnida and analyze their expression patterns on fed and not fed animals. **Methods:** The mRNA from the hepatopancreas of the scorpion *Tityus serrulatus* (fed and not fed), the spider *Nephilengys cruentata* (fed and not fed) and the harvestmen *Neosodocus sp.* (fed) were extracted and used to the synthesis of cDNA libraries which were submitted to a high-throughput sequencing on HiSeq Systems (Illumina). The generated transcripts were analyzed with Oases (EMBL-EBI) and the contigs were annotated. Lipase sequences were analyzed *in silico* in order to evaluate secretion (Signal IP, SOSUI), identification of catalytic residues. In order to validate the transcriptome data we determine the protein profile (proteome) from *T. serrulatus* and *N. cruentata.* Thehepatopancreaswere homogenized in Dounce homogeneizer and submitted to a cellular fractioning. After that, the protein profile was characterized on different cell fractions. We also determine the proteome from *N. cruentata* digestive juice. The colleted data was analyzed with Scaffold (Proteome Software) **Results andDiscussion:** Sequence alignment allowed the identification of 12 annotated lipases for *T. serrulatus* (6 PLRP2, 2 pancreatic TAG lipase [PnTAGLip.], 1 lysosomal [Lys.], 1 Gastric TAG lipase [GsTAGLip.] and 2 hormone-sensitive [hs.]), from which 4 are possibly secreted and presented all catalytic residues (2 PLRP2, 1 PnTAGLip. And 1 Lys); 11 sequences for *N. cruentata* (3 PLRP2, 6 PnTAGLip, 1 GsTAGLip and 1 hs), from which 5 are secreted and catalytic (1 PLRP2, 3 PnTAGLip. And 1 GsTAGLip); and 12 for *Neosodocus sp.* (5 PLRP2, 4 PnTAGLip, 2 GsTAGLip and 1 Lys), from which 4 are soluble and catalytic (3 PLRP2 and 1 GsTAGLip). The proteome analysis from *N. cruentata* digestive juice allow the identification of 2 lipases that align with high identities with lipases previously identified on transcriptome analyses: NcPTL01(59 kDa) and NcPTL02(58 kDa). Differential expression analysis indicated that, catalytic lipase, up or down regulation, are directed related to feeding conditions. Besides that, similar to insect lipases, possibly non-catalytic lipases have other physiological functions. We could validate that the NcPTL01 and NcPTL02 are expressed proteins acting on extraoral lipid digestion on*N. cruentata*.

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2.11New insights about the molecular physiology of digestion in the spider *Nephilengys cruentata* revealed by the combination of high throughput techniques

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Introduction: Next generation sequencing (NGS) and shotgun proteomic (SP) are two high throughput tools very efficient to a deep analysis in organisms with unknown genomes. Spiders have an elegant mechanism of digestion, combining extra-oral with intracellular digestion. The food is first liquefied (partially digested) outside the spider body by the digestive juice which is regurgitated into the prey. The final digestion will take place inside the cells from the midgut glands after pinocytosis. Nevertheless, few spider digestive enzymes were identified and sequenced and very little is known about the molecular aspects of this process. **Objectives**: Use high throughput techniques to understand the spider digestive process by the study of the molecular composition of the midgut and midgut glands (MMG) and the digestive juice (DJ) from the spider *Nephilengys cruentata* under different physiological conditions. **Methods**: The mRNA from the MMG of fastened and fed animals were submitted to a NGS analysis using Illumina® platform. SP experiments were done in a LTQ Orbitrap Velos after differential ultracentrifugation of the midgut glands from fastened or fed animals and the DJ was analyzed after different times of feeding .The proteins were searched using the software MASCOT (Matrix Sciences) and it was considered as a positive identification only the ones with at least two identified peptides (false discovery rate of 0.1%). **Results and Discussion**: About 12 million reads for each condition were assembled in 27,925 and 31,318 sequences in fastened and fed animals, respectively. One third of the contigs were associated with known proteins after a BLASTX analysis using the Uniprot database. A total of 1,150 and 556 proteins were identified in fastened and fed animals after the SP experiment, respectively, whereas 310 proteins were found in the DJ. The most abundant enzymes found in the later were astacin-like metallopeptidases with an impressive number of 26 different molecules. Other endo and exopeptidases, glycosidases and lipases were also identified. Besides that, proteins involved in the vesicular trafficking, immune system, cellular signaling as well as chaperons, toxins and peptidase inhibitors could also be detected in the DJ. Moreover, the differential ultracentrifugation procedure in the MMG cells brought the first insights about the subcellular location of digestive enzymes in different physiological conditions. These results showed, for the first time, that 1) DJ is a product from the MMG cells, 2) fastened spiders already contains the enzymes needed for the next predation event and 3) DJ has more complex functions than digestion.

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2.12 Activation of pro-SVMPs: Quantitative analysis of zymogens in venom gland extracts of *Bothrops jararaca*

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Introduction: Snakebitesare neglected diseases with high mortality rates in the world. In Brazil, numbers of accidents are above 25,000/year, mostly caused by *Viperidae* snakes. The venoms of these snakes comprise complex mixtures of molecules with various toxic activities. Snake Venom Metalloproteinases (SVMPs) are abundant in venom with important roles in the symptoms of envenomings. They are zinc-dependent hydrolases that share structural and functional domains with MMPs and ADAMs. These enzymes are synthesized as zymogens and the pro-domain is responsible for the inactivation of the proteolytic activity prior to secretion. **Objectives:**To understand the point in time when activation of SVMPs occurs, we aim to quantify the presence of zymogens and activated SVMPs in extractsof*B. jararaca*venom glandscollected at the peak of venom production (7 days after stimulus) and in quiescent form (40 days after stimulus).**Methods:** Quantitative analysis was carried out by ELISA sandwich assay in which levels of zymogens were calculated by detection of pro-domain and levels of activated SVMPs by the detection of mature jararhagin (Jar), a class PIII SVMP isolated from *B. jararaca* venom. The recombinant pro-domain of Jar (PD-Jar) was obtained in *Escherichia coli*and Jar as described previously (Paine et al, 1992).Antigens were detected by PD-Jar or Jar antibodies raised in mice and rabbits, followed by reaction with peroxidase conjugates specific to the secondary antibody. **Results and Discussion:**Production of recombinant PD-Jar resulted in a band of 21kDa, yielding 35 mg/L of cultureand purification of native Jar resulted in a 52 kDa band yielding 1.6 mg/100 mg crudevenom. For standardization of ELISA, we tested different microplates, concentrations and order of first and secondary antibodies. The best results were obtained with Maxisorb Nunc microplates, using 1:100 anti-PDJar antibodies raised in mice as first antibody and in rabbit (1:500) as secondary antibody (zymogen assay) and using 1:1000 anti-Jar antibodies raised in rabbit as first antibody and in mice (1:1000) as secondary antibody (assay of mature enzymes). In 10 µg of quiescent gland extracts, 291.5 ng of mature SVMP and 17.1 ng of pro-domain were detected, with the proportion of 5.84% molecules in the zymogen form. In 10 µg samples of 7 days extracts, 40.4 ng of active SVMP and 39.1 ng of pro-domain were detected, showing that 97% of SVMPs are still as zymogens. These data suggest that in the quiescent state SVMPs are mostly active, with their pro-domains processed and probably degraded. In the peak of venom production, SVMPs are majorly in the zymogen state. These data suggest that activation of SVMPs occurs gradually along the venom production cycle.

Supported by FAPESP

2.13 Kynuranic acid is the major component of the skin secretion of *Pipa carvalhoi*

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Introduction: The search of new molecules presenting relevant biological activities is constantly increasing. Innumerous animals have been studied, including amphibians that present promising molecules, such as alkaloids and steroids. **Objectives:** Isolate and characterize the major components of the skin secretion of *Pipa carvalhoi*. **Methods:** Skins secretion solutions from *P. carvalhoi* were obtained using two methods: chemically or mechanically stimulation. The first consist in the subcutaneous administration of norepinephrine followed by the animal submersion in 25mM ammonium acetate pH 7.0, for 30 min. For mechanical stimulation, animals were submerged in 25mM ammonium acetate pH 7.0 and massaged both in dorsal or ventral region of body. The skin secretion solutions from both methods were lyophilized, resuspend in appropriated buffer, filtered, analyzed and fractionated by Reverse Phase – High Performance Liquid Chromatography(RP-HPLC) and analyzed by Mass Spectrometry (LC-MS). The major peak present in this secretion was purified, submitted to structural characterization (NMR) and biological activities (antimicrobial, hemolytic and larvicide assays). **Results and Discussion:** Withoutanystimulation, *P. carvalhoi*secretes few molecules into its skin; apparently there is a constitutive level of molecules in the skin secretion, as assessed by chromatographic analyzes. Following stimulation, however, we can observe the presence of several peaks in this secretion, which have been grouped in 14 fractions, being fraction '8' the major peak of this secretion. LC-MS analyzes revealed that this fraction present only one molecule with m/z 188.036. Nevertheless, this fraction was not capable to reduce bacteria growth. Also, it exhibits no hemolytic effect and was not capable to kill or inhibit the metamorphosis of *Culex quinquefasciatus* and *Aedes aegypti* mosquito larvae. NMR analyzes shows that this molecule is the Kynuranic acid, a known metabolite of tryptophan. Moreover, chromatographic profiles of *P. carvalhoi* skin secretion were similar throughout different skins secretion collection events, even when employing different methodologies to obtain the material. In conclusion, Kynuranic acid is the major constituent of *P. carvalhoi* secretion; however, no biological effect could be associated with this molecule yet. Nevertheless, we hypothesize that once *P. carvalhoi* anurans live in aquatic environments, perhaps Kynuranic acid may act as a chemical communication molecule, signalizing other anurans before stressful events.

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2.14 Leukotriene (LT)-B4 and LT-A4-Hydrolase (LTA4H) in Collagen-Induced Arthritis (CIA)

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Introduction: The involvement of LTB4 and LTA4H in autoimmune inflammatory diseases is poorly understood, and the comparison about the relative efficiency of high performance liquid chromatography (HPLC) and enzyme immunoassay (EIA) to measure these parameters is not available. **Objectives:** To compare both methodologies, and to evaluate the relationship of LTB4 and LTA4H with the development of experimental arthritis in rats. **Methods:** Healthy rats (C) submitted to CIA induction which developed (arthritic - AR) or not (resistant - RE) the disease were used. Plasma (PL), synovial fluid (SY) and soluble (SF) and solubilized membrane-bound (MF) fractions from synovial tissue (ST) and peripheral blood mononuclear cells (PBMCs) were subdivided to be incubated with LTA4 or with the diluent of LTA4. After incubation, part of these samples was applied directly in EIA kit and other part, after passage in C18 Sep-Pak micro column, applied in HPLC. An isocratic run was adopted and the amount of LTB4 was determined by interpolation of the area of the peaks, monitored by λ=270nm, obtained in the same RT of standard LTB4. The corresponding peaks to RT were collected and their LTB4 amounts were also measured by EIA. LTA4H activity was expressed by the measurement of the LTB4 formation per unit of time, in samples incubated with LTA4, from which the content of endogenous LTB4 was subtracted. **Results and Discussion:** LTB4 quantification by EIA provides values with a high degree of correlation with the peak's area of HPLC. Compared to HPLC, EIA does not need cleaning steps, thereby simplifying the analysis and shortening its time course. LTB4 values from EIA obtained for collected peaks in HPLC are not correlated with values of LTB4 directly measured by EIA or peak's area by HPLC, indicating that sample's processing required for HPLC is able to alter the immunogenic integrity of LTB4. Compared to C, in SY and MF-PBMCs of RA and RE and in MF-ST of RE, changes on LTB4 and LTA4H activity, measured by EIA, are parallel and positively related, confirming in these cases the primordial role played by LTA4H in the biosynthesis of LTB4. However, in plasma, SF-ST and SF-PBMCs of RA and RE and in MF-ST of AR that correlation is not detected, suggesting in these cases that changes on LTB4 formation involve other steps of LTB4 biosynthesis and degradation without participation of LTA4H. The endogenous content of LTB4 does not also have a unique sense of change in all examined samples of AR and RE in relation to C. Data show significant differences for LTB4 content and LTA4H activity among C, RA and RE, suggesting the involvement of both in the experimental development of this disease.

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2.15 Scorpion Immune System: *Tityus serrulatus*

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Introduction: Antimicrobials peptides (AMPs) are important components of the immune system of vertebrates and invertebrates and, in the last 20 years, many of these molecules were isolated and characterized in several organisms. The immune system of the invertebrates does not possess immunologic memory and no immunoglobulin, in that way the defense against microorganisms is accomplished by hemocytes and other substances as the antimicrobials peptides. This study, besides supplying a better understanding about the immune system of the arachnids and other arthropods, can also contribute to the production and use of new drugs in medicine and agriculture, through the description and characterization of new molecules with antimicrobial activity. In scorpion hemolymph were described AMPs in *Androctonus australis*,*Leiurus quinquestriatus* and *Centruroides limpidus*. The characterization of AMPs in this group would be of great value to understand the evolutionary aspects of innate immunity in arthropods.**Objectives**: The objective of this study was to identify AMPs in the hemolymph from the scorpion *Tytius serrulatus*.**Methods**:Hemocytes were submitted to acid extraction and fractionated in two steps. For the first step, a C18 Sep Pak column was used in three stepwise elution with 5, 40 and 80% acetonitrile (ACN) in trifluoroacetic acid (TFA) 0.05%. In the second step, the Sep-Pak fractions were concentrated in a vacuum centrifuge, reconstituted in TFA 0.05% and applied to a reversed phase chromatography on a semi-preparative C18 Jupiter column. The elution was performed for different linear gradients of ACN in TFA 0.05% over 60 min at a flow rate of 1.5 mL/min. The column effluent was monitored by absorbance at 225 nm and the antimicrobial activity was determined by liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria Micrococcus *luteus* and yeast *Candida albicans*. The active fractions were analyzed by Mass Spectrometry.**Results and Discussion:** After the RP-HPLC of the hemocyte acid extract (ACN 40%),we have detected antimicrobial activity in seven fractions that presented activity against *M. luteus*A270, and two of these fractions also presented activity against *E. coli*SBS36. After the study with the ESI-MS we find that the fractions that present activity against the microorganism used in the study, not presents homogeneity. It is necessary other steps of the purification for to homogeneity. The purification and characterization of these peptides are still in progress.

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2.16 New sources of α-L-fucosidases: spiders and scorpions. Identification,sequencingand characterization

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Introduction: Fucosidases are glycosyl hydrolases which cleave exo or endo glycosidic bound of fucosilated proteins, lipids, carbohydrates or sulfated polymer of fucose. Fucosylated glycans are heavily involved in many physiological processes, including antigenicity and immune responses, signal transduction, and the adhesion processes in pathogens. On the other hand, fucosidases are involved in a series of physiological process to remove these fucose residues. Fucosidase expression may be up or down regulated in some pathological conditions as in gastric diseases or in cancer. Besides that, α-L-fucosidases are also able to transglycosilate. New sources of α-fucosidases are interesting to the study of fucosidase specificity and mechanism. Arachnida species as spiders, scorpions and ticks present extra and intracellular digestion and these organisms have α-L-fucosidases as digestive enzymes. **Objectives**: Identify and characterize α-fucosidases from the spider *Nephilengys cruentata* MMG and *Tityus serrulatus* MMG by transcriptomic analysis using new generation sequencing (Illumina system) and shotgun proteomic analysis of spider and scorpion and by kinetic characterization using as substrates, fucoidan or 4-Methyl-umbellipheryl-α-L-fucoside (MUFUC) and inhibitors, fucose and deoxy-fuconojirimycin. **Methods:** Females of *Nephilengys cruentata* and *Tityus serrulatus* were immobilized on ice and their digestive tracts were isolated and homogenized in cold MilliQ water. Fucosidase activity was measured using MUFUC as substrate. Homogenized sample was then submitted to 30% ammonium sulfate fractionation at 4°C. Soluble fraction was applied into a Hitrap Butyl column and eluted with a linear decreasing ammonium sulfate gradient. Tissues were homogenized in Trizol (Invitrogen) for transcriptomic. **Results and Discussion**: One digestive fucosidase was identified by transcriptome and proteome at the MMG from *Nephilengys cruentata* (NcFuc) and two were found at *Tityus serrulatus* MMG (TsFuc56 and TsFuc52). All new fucosidases have the catalytic residues, the hydrophobic pocket and residues involved in substrate binding. Gel filtration on a Superdex G75 indicated a molecular mass of 230 kDa for Ncfuc evidencing an oligomerization process similar to other α-L-fucosidases. Ncfuc hadn´t hydrolyzed fucoidan in the conditions tested till now but presented a Km of 12.9 ± 1.7 μM to MUFUC and a Vmax of 0.74 mU. Fucose and fuconojirimycin are competitive inhibitors of Ncfuc presenting Kis of 52.4 μM and 3.8 pM respectively. These enzymes are being cloned and will be expressed in order to further characterize them.

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2.17 Unraveling processing and activation of Snake Venom Metalloproteinases.

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Introduction: Snake Venom Metalloproteinases (SVMPs) are abundant enzymes in *Bothrops* venoms responsible for local and systemic symptoms of human envenoming. These enzymes are synthesized as zymogens and the enzyme activation is regulated by hydrolysis of the pro-domain, as occurs in MMPs and ADAMs. However, it is still unknown how and where processing of the pro-domain and activation of SVMPs occur. **Objectives:** Considering the importance of processing for modulating the activity of this class of toxins, our aims are to identify in which cellular or extracellular compartment of the venom gland activation of SVMPs occurs. **Methods:**The cDNA fragment coding for the pro-domain of jararhagin (PD-Jar), a SVMP prevailing in the venom of *Bothrops jararaca,* was cloned into pAE vector and expression obtained in *E. coli.*Recombinant PD-Jar was used to immunize mice. Anti-PD-Jar antibodies were used in the identification of pro-domains by ELISA and Western-blotting (WB)in samples of venom and gland extractscollected in different periods after venom extraction (stimulus of venom production cycle). Proteins detected by WB were separated by immunoprecipitation and characterized by mass spectrometry. The location of pro-domains within the venom secretory cells wasaccessed by immunofluorescence and immunoelectronmicroscopy of gland tissues.**Results and Discussion:**Anti-PD-Jar antibodies reacted with bands of 22 and 45 kDa in venom samples collected 4, 7 and 10 days after extraction; bands of 22, 45 and 65 kDa in venom collected from the lumen of the glands 7 days after milking and bands of molecular masses above 45 kDa from gland extracts all along the cycle, but with higher intensity on days 4, 7 and 10 after milking. Most proteins present in these samples were characterized by mass spectrometry as zymogens of the three classes of SVMPs.The results of immunofluorescence microscopy show positive staining for pro-domain in the apical surface of secretory cells and by immunoelectron microscopy the presence of pro-domains was majorly detected in their secretory vesicles.This data suggest that SVMPs are secreted as zymogens and processing occurs as soon as they reach the lumen of venom gland. Pro-domain is degraded after processing and we hypothesize that the resulting peptides should act to regulate the activity of SVMPs within the venom gland, together with the acidic pH of the lumen and the tripeptide of pyrol-glutamate existing in it, thus contributing to the preservation of tissues by reducing proteolytic activity within the venom gland.

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2.18 Antimicrobial peptide with antiviral activity: Rondonin

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Introduction: A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. We found an antifungal peptide in the plasma of *Acanthoscurria rondoniae* and this molecule was characterized by Mass Spectrometry like a single molecule with 1,236.405 Da. This peptide has been submitted by "de novo" sequencing, elucidating its primary structure: IIIQYEGHKH, which showed similarity with a fragment of subunit "D" haemocyanin and nominated rondonin. This spider can live in different kind of ambient and it's regularly exposed to environmental contaminants. **Objectives:** The objective of this study was verifying the cytotoxicity and activity of rondonin against human viruses.**Methods:** Synthetic rondonin was obtained by China Peptides at degree 98%. This peptide was evaluated against *Candida albicans* MDM8 at different pH (4-8). VERO and MDCK cells were grown on plastic T-flasks or on multi-well plates using Leibovitz-15 (L15) medium containing 0.9 $g.L^{-1}$ of D-galactose, 0.3 $g.L^{-1}$ glutamine supplemented with 5% fetal bovine serum (FBS). Influenza (H1N1) and Measles (Edmonston) viruses were used to determine the antiviral activity of rondonin. VERO cells were infected with measles and MDCK cells with influenza virus. The cells cultures were seeded at a concentration of $5x10^4$cells mL^{-1} on 96-well plates. After 24 hours, the rondonin was added to the culture at 9 μM and after one more hour these cells were infected with viruses at dilution rates of 10^{-1} to 10^{-10}. The 96-well plates containing H1N1 and measles were then incubated at 37°C for 3 days and 7 days respectively. Virus titers were determinate by monitoring the cytopathic effect in an endpoint dilution assay. The cytotoxic effects of the rondonin were assessed by using a standard VERO cell assay and after 24 hours the supernatant were removed and the remaining living cells were counted after being fixed and stained with crystal violet (0.2% in 20% methanol). **Results and Discussion:** According our results, rondonin was pH dependent; this peptide has the best activity in acid pH with 25 μM, no cytotoxic at 100 μM against VERO cells and showed activity against measle virus at the 10^{-2} dilution. This result led us understand why this animal can live in theses environment and survive for a long time without change in evolution.

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2.19 Bradykinin potentiating peptide (BPP) from *Bothrops jararaca* as a cell penetrating peptide (CPP)

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Introduction: Bradykinin potentiating peptides (BPPs) are widely studied and known peptides present in the snake´s venoms, as*Bothrops jararaca*. Typical BPPs are proline (P) rich, characterized by the presence of PP in the C-terminal. Cell penetrating peptides (CPPs) are peptides that have the ability to pass through the cell membrane, and have been developed as a drug delivery. These peptides typically have a large amount of proline residues. **Objectives:** The aim of this study was to evaluate the activity of BPPs from *Bothrops jararaca* venom, rich in proline, as a CPP. **Methods:** The *Bothrops jararaca* venom was fractionated by ultracentrifugation, using a cutoff of 3 kDa, in order to obtain a fraction rich in peptides. This fraction was purified by RP-HPLC (C18 column) and the peptides were assayed on liposomes, to evaluate if BPPs have the ability to enter in synthetic vesicles. Peptides were incubated with liposomes for 30 minutes. Then, liposomes were lysated with acetronitrile and formic acid, and the content inside was analyzed by HPLC and mass spectrometry. **Results and Discussion:** It was possible to find two peptides that could enter in the liposomes. One of them is one BPP named 13A, with molecular mass of 1369.5 Da and one signature ion of m/z 213 (corresponding to the y-ion of the C-terminal PP fragment). The other peptide has a molecular mass of 1356.7 Da, not described in the literature and not sequenced yet, but with an ion 213 m/z, typical of BPPs. Although the sequence and activity of this peptide is unknown, it has already been found, both in female and male *Bothrops jararaca* snakes. In conclusion, we have found two BPPs that could pass through liposomes, being candidates to CPPs. Now, both peptides will be tested on cell cultures, in order to evaluate the activity over cells membranes.

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2.20 The cyclic nitroxide tempol inhibits murine hepatitis vírus, strain A59 (MHV-A59) proliferation by inhibitory effect on cyclooxygenase 2 production

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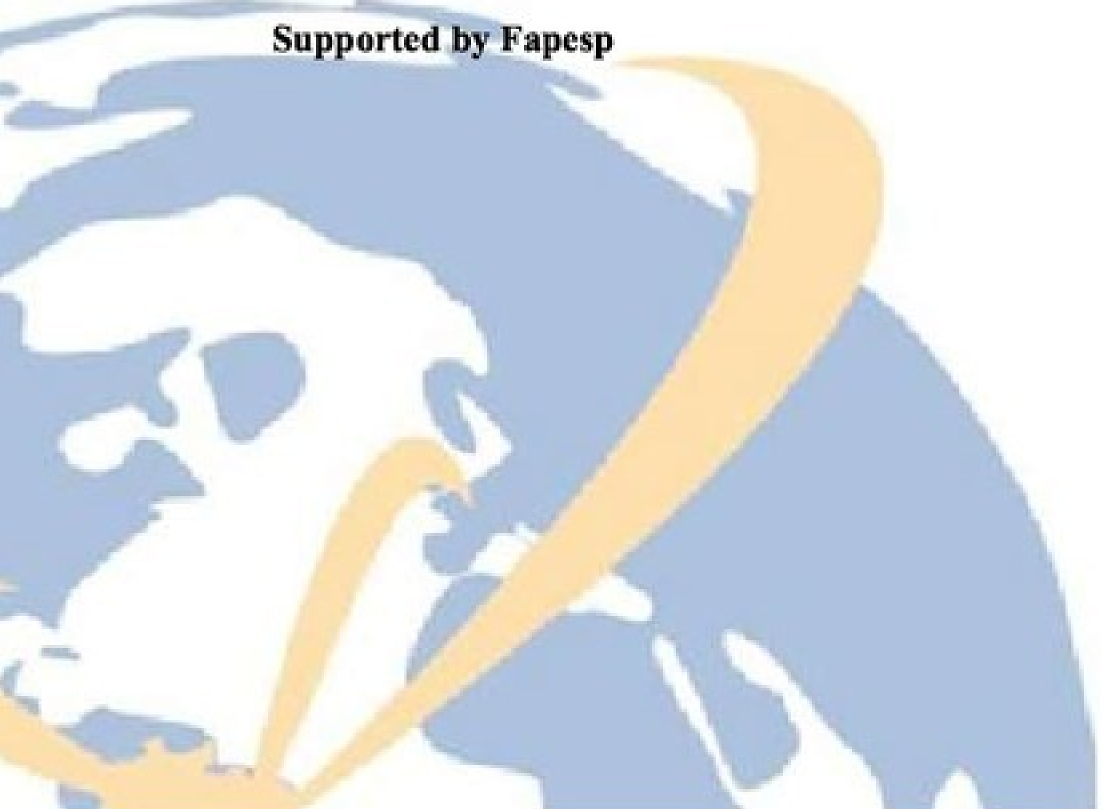
Introduction: Recently, we showed that tempol greatly attenuates multiple sclerosis in an experimental model of the disease induced by the murine hepatitis virus, neurotropic strain MHV-A59. In parallel, tempol decreased the viral titers in the CNS of treated mice. **Objectives:** Considering the lack of knowledge about the effects of tempol on viral replication, we investigated the phenomenon in cell cultures. **Methods:** Tempol treatment was performed in astrocytoma DBT cells before and after virus adsorption. The cultures were infected with MHV-A59 (MOI 0.04), treated with 500 μM tempol and incubated at 37°C for 8h. The number of MHV-infected cells was determined by counting the number of syncytia. The expression level of the N gene of MHV-A59 was determined by Taqman RT-PCR. The cytotoxicity of tempol was checked by the Trypan Blue Exclusion Test. Remaining concentrations of tempol were monitored by EPR spectroscopy.**Results and Discussion:** Significant reduction of viral production (54 ± 6.8%, p □0.007) and of viral RNA synthesis (45.67 ± 6.50%, p<0.029) were observed when the cells were treated with tempol after viral adsorption. Marginal differences were observed when the cells were treated before viral adsorption as shown by the relative % of infected cells (90.31 ± 3.71%, p = 0.2) and the % of viral RNA (91.33 ± 13.22%, p = 0.38. Since prostaglandins, generated by cyclooxygenases, have been shown to participate in the regulation of virus replication and in the modulation of inflammatory responses following infection, we analyzed the levels of COX-2, COX-1, iNOS and Nrf2 proteins by western blotting or slot blot. At 8 hpi, these experiments showed a markedly inhibitory effect on COX-2 expression, a decrease in iNOS and a marginal increase of Nrf2 levels in lysates of cells treated with tempol compared with mock-treated MHV-A59 infected cells. The COX-1 protein levels remained unchanged. In DBT cell cultures infected or not with MHV-A59 tempol are metabolized to products that are not oxidized by ferricyanide. These results suggest that tempol at a non cytotoxic concentration interferes with viral RNA synthesis and with production of infection particles when added after virus adsorption by its inhibitory effect on COX-2 expression. Our findings also shown that nitroxides have potential antiviral activity and that tempol may be an usefull adjuvant in the treatment of viral infection.

Supported by Fapesp



2.21 Prospecting for bioactive molecules: bacilli Doderlein

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Introduction: Some microorganisms have the ability to produce substances that may influence the development of other microorganisms. Since the early 50's reported the ability of various species of bacteria of the genus Bacillus to produce substances with antimicrobial activity, among these are reported to subtilisin, proteases and the termolisins. When it comes to antimicrobials the greater emphasis is given the bacteriocins. These are defined as Antimicrobial Peptide that destroy or inhibit the growth of other bacteria. The structural function, biosynthesis and mode of action of some bacteriocins, many aspects of these compounds remain unknown. The normal vaginal microbiotic is rich in lactobacillus peroxide producers, which form lactic acid from glycogen. These organisms constitute 98% of vaginal microbiotic, inhibiting the growth of opportunistic organisms. The Bacilli of Doderlein feature with the product of hydrogen peroxide metabolism and other organic metabolites as bioactive molecules, becoming of great interest for studies. **Objectives:** The present study aims at purification and characterization of bioactive molecules from Bacilli of Doderlein. **Methods:** we conducted a growth of microorganisms from a Pre-inoculum, after incubation was centrifuged and supernatant discarded and every pellet was placed in saline. The pellets were submitted to acid extraction and fractionated in two steps. For the first step, a C18 Sep Pak column was used in three stepwise elution with 5, 40 and 80% acetonitrile (ACN) in trifluoroacetic acid (TFA) 0.05%. In the second step, the Sep-Pak fractions were concentrated in a vacuum centrifuge, reconstituted in TFA 0.05% and applied to a reversed phase chromatography on a semi-preparative C18 Jupiter column. The elution was performed for different linear gradients of ACN in TFA 0.05% over 60 min at a flow rate of 1.5 mL/min. The column effluent was monitored by absorbance at 225 nm and the antimicrobial activity was determined by liquid growth inhibition assay against bacteria and yeast. The active fractions were analyzed by Mass Spectrometry.**Results and Discussion:**After the RP-HPLC of the acid extract (5, 40 and 80% ACN), we have detected antimicrobial activity in fifteen fractions. All fractions presented activity against *Candida albicans, Micrococcus luteus, Escherichia coli and Aspergillus niger*. After the study with the ESI-MS we find that the fractions that present activity not presents homogeneity. The purification and characterization of these peptides are still in progress. To better characterize the fractions that presented activity, will necessary to do mass spectrometry and database analysis to find similarity with other bioactive molecules.

Supported byFAPESP e CNPq

2.22 Antiviral effects of bufotenine against EMC and H1N1 virus

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Introduction:*Bufo*genus contains a large number of alkaloids in their skin secretion, such as bufotenine, a tryptamine alkaloid, used as a defense mechanism due to its toxic property. This alkaloid is widespread throughout Nature, being also found in the Leguminosae family. Itsstructure is related to psilocin and DMT, which are known hallucinogens, as well to the neurotransmitter serotonin.**Objectives**: Once bufotenine was effective against rabies virus infection in mammalian cells culture, in this work we tested this alkaloid against H1N1 and EMC virus.**Methods:***Rhinella jimi* skin secretions were collected through mechanical stimulation. A liquid-liquid partition (H_2O:CH_2Cl_2) was performed and analyzed by RP-HPLC in a C18 column with a linear gradient of B over A (10% to 70%) in 35 min and flow rate of 1.7 $mL.min^{-1}$. Fractions were collected and submitted to mass spectrometry (MS and MS^2 ESI-IT-TOF) analyses. The fraction containing bufotenine was purified using a C18 column, with a linear gradient of B over A 13% to 15% in 15 min and flow rate of 1.1 $mL.min^{-1}$, at 4 °C. Bufotenine peak was collected, dried and submitted to ^{1}H-NMR spectroscopic analyses.The extraction of bufotenine from *Anadenanthera colubrina* seedswas performed as described by Stromberg (1954), following RP-HPLC analysis, in a C18, with a linear gradient of B over A 0% to 100% in 20 min and flow rate of 1 mL/min. Bufotenine peak was collected and dried for mass spectrometry and biologic assays.The antiviral activity was evaluated with picornavirus (EMC) in L929 cells and influenza virus (H1N1; A/SP/1/78) in MDCK cells. **Results and Discussion:** RP-HPLC analysis of the aqueous partition of *Rhinela jimi* skin secretion showed the presence of 5 major HPLC peaks. MS and MS^2 analysis compared with already published data showed the presence of two indole alkaloids contained in this fraction: bufotenine (205 m/z) and 5-HTQ (219 m/z). These two compounds were separated by RP-HPLC yielding pure bufotenine, as confirmed by NMR analyses. Bufotenine from *Anadenanthera colubrina* seeds were purified after only one chromatographic step, following the acetone extract, being the major peak on the RP-HPLC chromatogram and appearing pure on mass spectrometric analyses. Initial antiviral experiments showed that bufotenine was able to reduce the viral cytopathic effects of both EMC and H1N1 virus, decreasing at least 1.000 times the EMC virus title and 128 times the H1N1 virus title. These are preliminary results and bufotenine will be retested for biological activity against these and others virus, using experiments that will lead us to understand the antiviral effects of this alkaloid.

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3. Pharmacology

3.01 Hypothalamic obesity induced by neonatal administration of monosodium glutamate changes melatonin synthesis profile in adult rats

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Introduction: The pineal gland through the rhythmic synthesis of the hormone melatonin synchronizes the internal milieu. Melatonin is synthesized only at night and has a role in the regulation of circadian rhythms as well as in several physiological systems. **Objectives**: The purpose of this work was to analyze the nocturnal melatonin content and the arylalkylamine-N-acetyltransferase (AANAT) enzyme activity in the pineal gland of males and females rats with hypothalamic obesity induced by neonatal administration of monosodium glutamate (MSG). MSG induces metabolic alterations when neonatally administered characterized by obesity, growth deficiency, insulin resistance, as a consequence of arcuate nucleus lesion. **Methods**: Wistar rats were injected (i.p) with MSG (4mg/g/day) or saline (0,9%) for 7 days, from the second to the eighth postnatal day. Weight and naso-anal length were measured one day before sacrifice. The animals were euthanized with 2, 3, 4, 5 and 6 months of age in order to isolate the visceral adipose tissues (retroperitoneal and periepididymal) and the pineal glands. **Results and Discussion**: It was observed the characteristic obesity described in the literature, with an increase invisceral white adipose tissue weight for both males and females. The naso-anal length did not change significantly, but the weight / length ratio (Lee Index) was considerably greater in the MSG groups when compared with controls. Melatonin synthesis profile was different between the two groups, in spite of the fact that both exhibited circadian rhythms. MSG induced a greater melatonin synthesis at ZT 15 (3h after lights off) and the same was observed for AANAT activity, for both males and females. The cosinor analysis showed a phase advance in melatonin synthesis for the males at 4 and 5 months of age. Metabolic alterations induced by neonatal MSG administration led to melatonin synthesis changes that could interfere with circadian rhythms andenergy metabolism regulation.

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3.02 Involvement of cytoskeleton in crotalphine antinociceptive effect: *in vivo* and *in vitro* assays

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Introduction: Crotalphine, a peptide first identified in the venom of the snake *Crotalus durissus terrificus*, induces potent and long lasting antinociceptive effect in different models of acute and chronic pain. It's mechanism of action is not completely understood, but involves activation of peripheral κ- (PGE_2- induced hyperalgesia model) or κ- and δ- (neuropathic and cancer pain models) opioid receptors, followed by activation of L-arginin/NO/cGMP pathway and opening of ATP-sensitive K^+ channels. Crotalphine does not bind directly to opioid receptor, but increases dynorphin-A release, which mediates its antinociceptive effect. The cytoskeleton is a dynamic net of proteins that can regulate the organization and expression of membrane proteins such as opioid receptors, and modulates the intracellular signaling pathways activated by these receptors. **Objectives:** The aim of this work is to characterize the role of cytoskeleton in crotalphine antinociceptive effect.**Methods:** Latrunculin B or cytochalasin D was used to disrupt microfilaments; nocodazole or colchicine was used to disrupt microtubules; acrylamide was used to disrupt intermediate filaments. Male Wistar rats were treated with intraplantar administration of PGE_2, cytoskeleton inhibitors (90 min after PGE_2) and crotalphine or κ opioid receptor agonist U50,488 (30 min after cytoskeleton inhibitors). The nociceptive threshold was determined 3, 4 and 5 hours after PGE_2 injection by the paw pressure test. Crotalphine-mediated release of endogenous opioid peptides was determined in the plantar tissue of PGE_2 and cytoskeleton inhibitors-treated rats, using ELISA technique. All the procedures were conducted in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals, and were approved by the Animal Ethics Committee of the Butantan Institute (protocol number 855/11).**Results and Discussion:** PGE_2-mediated hyperalgesia was blocked by crotalphine or U50,488. All cytoskeleton inhibitors decreased crotalphine antinociceptive effect and blocked the effect of the U50,488. In PGE_2-treated rats, crotalphine increased dynorphin-A release, and cytoskeleton disruption inhibited this effect. The results demonstrated that microfilaments, microtubules and intermediate filaments are key elements for the antinociceptive effect of crotalphine, since the selective disruption of these elements prevented or blocked crotalphine antinociceptive effect and crotalphine-mediated dynorphin-A release.

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3.03*In vivo* effects of four peptides isolated from the venom of *Tityus serrulatus:* evaluation of nociception effect and leukocytes rolling

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Introduction:Accidents caused by scorpions represent an important public health problem in Brazil. Most of the fatalities resulting from accidents are caused by *Tityus serrulatus*. Despite of the potency of its venom, what is currently known about its molecular components is restricted to neurotoxins acting on ion channels, and other biologically active peptides are poorly explored. The *T. serrulatus* venom (TsV) peptides interact, in vitro, with endopeptidases EP 24.15 and EP 24.16, human metallopeptidases involved in a series of processessuch asthe perception of pain, the cardiovascular and renal homeostasis. **Objective**:To verify nociceptive effect andleukocytes rolling*in vivo*of four synthetic peptides (K1, K2, F, Y) derived from the low molecular weight fraction of TsV selected by interaction with oligopeptidases*in vitro*.**Methods**: To evaluate antinociceptive activity, the peptides were evaluated in the rat paw pressure test described by Randall &Selitto (1957).Hyperalgesia was induced by injection in the hind paw of 0.1 ml saline containing carrageenin (200µg). Hypernociceptive threshold was measured before injection of carrageenin and after three hours, with or without co-administration of peptide (20µg). To analyze the rolling of leukocytes, the synthetic peptides of *T.serrulatus* (20 µg / 0.1 ml) or saline (0.1 ml) were applied topically on mice cremaster muscle microcirculation. The preparation was examined under a light microscope with 10X objective, coupled to a camera to capture images. The rolling was counted over a 10 min after exposed muscle, and 30 minafter application of peptides on the microcirculation in the range of 10 min. **Results and Discussion:** To evaluate the antinociceptive activity peptides were tested in the rat paw pressure test and three of them were not capable to causing antinociception in animals. On the other hand, peptide F induced antinociceptive effect compared to control carrageenin, a hyperalgesic inductor. Further studies will be required to the understanding of this effect, which may be related enzymes employed in the isolation of this peptide.In tests carried out on changes in leukocyte-endothelial interactions induced by peptides in the microcirculation of the cremaster muscle suggest that the peptide K1 is involved with increased rolling of leukocytes after application. The causes of this effect have not been elucidated. The other peptides are being tested.

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3.04 Involviment of TRPV1 receptors in theanalgesic effect ofBDS391 on capsaicin-evoked thermal hyperalgesia

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Introduction: BDS 391 is a low molecular weight (~390 Da) and non-peptidic compound purified from the Brazilian sea anemone *Bunodosoma cangicum* venom. Studies on the structure of BDS 391 have demonstrated that this compound is composed of a bromoindole group connected to histidine. Interestingly, this compound induces a potent analgesic effect mediated by activation of 5HT3 receptors; however this compound does not directly activate these receptors. Data from the literature have indicated an interaction between 5HT and TRPV1 receptors.**Objective:** The aim of the present work is to further characterize the action of BDS 391 on TRPV1 channels, evaluating the effect of the compound on: (a) capsaicin (TRPV1 agonist)-evoked thermal pain and (b) capsaicin-stimulated calcium influx.**Methods:** Male Wistar rats and mice C57BL/6 were used. Thermal hyperalgesia was induced by i.pl. injection capsaicin (1nmol/50µL) in one of the hind paws. Thermal hyperalgesia was evaluated 15, 30 and 60 min. after capsaicin injection and determined by withdrawal latency (s) of the paw to heat stimulation. BDS 391 (75pmol/50µL) or saline were administered i.pl. 15 min before capsaicin. The effect of BDS 391 on capsaicin-stimulated calcium influx was evaluated by ratiometric $[Ca^{2+}]i$ measurements in DRG neurons and HEK293t cells transiently transfected with human TRPV1.All experiments were previously approved by CEAUIB – Butantan Institute. **Results and Discussion:**Administration of capsaicin decreased paw withdrawal latency that lasted for 1h. BDS 391 inhibited the capsaicin-evoked thermal hyperalgesia. BDS 391 did not change, per se, the calcium influx in both systems. On the other hand, this compound (1.5 µM) reduced by 50% the capsaicin-induced calcium influx in DRG. Also, when co-applied with capsaicin, BDS 391 (1.5 nM and 1.5 µM) inhibited capsaicin-induced calcium influx in hTRPV1-transfected cells. These results showed that BDS 391 induces antinociceptive effect in the capsaicin-evoked thermal pain. Capsaicin, viaactivation ofTRPV1 channels, causes the influx ofcalcium, which may contribute to thermal hyperalgesia. TheBDS391 is able of inhibiting calcium-influx caused by activation of TRPV1 channels, which may contribute to its antinociceptive activity.

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3.05 Assessment of the neurotoxic effects the intrahippocampal injection of three toxins isolated from *Tityus bahiensis* scorpion venom

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Introduction: In Brazil, *Tityus* scorpions from Buthidae family are the main responsible for accidents in humans and are considered the scorpions of greater medical importance. The scorpion venoms consist of a complex mixture of active components, being the neurotoxins the main toxic elements. Few studies are devoted to evaluate the actions of the venom of *T. bahiensis* mainly on the central nervous system. **Objective:** To evaluate the changes on the behavioral and electrographic activity parameters, after intrahippocampal injection of 3 toxins isolated from *T. bahiensis* scorpion venom. **Methods:** The procedures were approved by the Ethics Committee on Animal Use (CEUA / IBU protocol 870/11). We used male Wistar rats, weighing 240-260g. To obtain isolated toxins, *T. bahiensis* crude venom was fractionated by gel filtration, and the five pools obtained were dialyzed. The activities of these pools were tested. The pool 2 showed greatest potential to promote changes. This pool was chromatographed on HPLC equipped with a C18 analytical column and the 3 peaks obtained were employed in the following tests. The animals were submitted to stereotaxic surgery and cannulas and electrodes were implanted in the CA1 hippocampal area. The animals were divided into 4 groups (n=6) which received intrahippocampal injection of the Ringer's solution (1µL control group) or toxins TbII-I, TbII-II or TbII-III (2µg/µl experimental groups), respectively. After electrographic recording (AEC) and behavioral observation, the animals had their brain removed and processed for histopathological analysis of CA1, CA3 and CA4 hippocampal areas, ipsilateral (i) and contralateral (c) to injection. The data were analyzed by Fisher test (behavioral activity and AEC) and by t Student test (histological evaluation). **Results and Discussion:** The animals from TbII-I and TbII-II groups showed significant changes in behavioral parameters as respiratory distress, myoclonus and WDS. With regard to AEC parameters it was observed spikes and strong discharge. The toxin TbII-III did not cause significant changes in these parameters. Concerning the histological analysis, the three toxins injected reduced the number of viable cells in CA1, CA3 and CA4 hippocampal areas, both ipsilateral (side of injection) and contralateral. The changes caused by the toxins in the analyzed parameters indicate that these toxins have an epileptogenic potential, as well as are able to cause neuronal injury in hippocampus.

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3.06 Lipid body formation induced by CB, a phospholipase A_2 from *Crotalus durissus terrificus* (C.d.t) venom: signaling proteins involved and implication in prostanoids synthesis

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Introduction: CB is a subunit of crotoxin, the main component of C.d.t venom. CB is myotoxic, neurotoxic and affects diverse functions of macrophages (MΦs). Upon activation MΦs exhibit an increased number of lipid bodies (LBs), which are relevant organelles for lipid metabolism and synthesis of inflammatory mediators. **Objectives:** To investigate the effects of CB in MΦs, evaluating: i) LBs formation and the signaling proteins involved in this effect; ii) ultrastructural alterations of MΦs; iii) PLIN2 distribution and protein expression; iv) COX-1, PGE_2 and PGJ_2 subcellular distribution. **Methods:** Murine thioglycolate-elicited MΦs were incubated (1-12h) with either RPMI (control) or non-cytotoxic concentrations of CB (CEUAIB 846/11). LBs formation was evaluated by osmium tetroxide staining followed by contrast phase microcopy analysis. Ultrastructural cell modifications were evaluated by transmission electron microscopy and participation of signaling proteins determined by pharmacological interferences. PLIN2 protein expression was determined by W.blotting and distribution of PLIN2, COX-1, PGE_2 and PGJ_2 by imunofluorescence assay followed by confocal microscopy analysis. **Results and Discussion:** Incubation of MΦs with CB (0.2 to 0.8 μM) significantly increased LBs numbers (1 up to 12 h). Ultrastuctural analysis revealed the presence of weakly electrondense LBs, with some LBs in association with enlarged ER. Moreover, CB increased PLIN2 recruitment and protein expression at 1, 3 and 12h. Treatment of MΦs with compound H7, a PKC inhibitor, or LY294002, a PI3K inhibitor, or JNK inhibitor, or U0126, a MEK1/2 inhibitor, abolished LB formation as well as PLIN2 protein expression and recruitment. This indicates participation of all these signaling pathways in CB-induced LBs formation and PLIN2 recruitment and expression. Confirming these findings, phosphorilation of those signaling proteins was detected 5 min after stimulation with CB. Increased COX-1, PGE_2 and PGJ_2 pools colocalized to LBs were found in CB-stimulated cells suggesting that LBs may constitute relevant intracellular sites of storage and/or synthesis of prostanoids upon CB stimulus.

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3.07 Effects of the combination of ultrasound and low power laser therapy in nociception and functional recovery of rats with peripheral nerve injury

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Introduction: Traumatic injuries of peripheral nerves are common and can lead to the loss of nerve function and pain. The clinical use of both therapeutic ultrasound and low power laser has evidenced to contribute to the nerve function recovery and cause analgesia. However, there are no experimental studies evaluating the effectiveness of the combined application of these resources in injury recovery. **Objectives:** The aim of this study was to investigate, in rats, the effect of the combined application of these resources in nociception and functional recovery of injured sciatic nerve. **Methods:** Male Wistar rats (160-180g, Butantan Institute, CEUAIB protocol number 820/11) were subjected to sciatic nerve injury, performed by clamping (static load of 5000 g, applied for 5 min). The animals were treated with association of terapeuthic ultrasound (UST - dosage of 1.0 W/cm2) and Laser (LBP - dosage of 6J) on the first postoperative day lasting for the 21 consecutive days. To evaluate the functional activity, it was used the sciatic functional index (SFI). For assessment of mechanical hyperalgesia, the rat paw pressure test was used. The evaluation of thermal hyperalgesia was performed by plantar test. The allodynia was assessed by quantitative assay, in response to tactile stimulation applied to the rat paws. To better characterize this injury model, pharmacological treatments were carried out with morphine 5 mg/kg s.c. and indomethacin 4 mg/kg i.v.**Results and Discussion:** Nerve crushing causes mechanical and thermal hyperalgesia, mechanical allodynia, and motor dysfunction that last for 21 days. Histopathological changes (significant decrease in myelinated fibers and uneven fiber pattern distribution) were also detected. Administration of indomethacin and morphine partially inhibited or blocked, respectively, nociception without interfering with sciatic functional recovery. The application of ultrasound (1.0 W/cm²) and laser (6 J) caused antinociception and partial nerve function and histopathological changes recovery. In conclusion, the combined application of ultrasound and laser is effective in controlling neuropathic pain resulting from sciatic nerve crush and in accelerating nerve regeneration.

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3.08 The role of sympathetic outflow in the mouse submandibular and parotid glands: a comparative proteomic study

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Introduction: Data in literature show that sympathetic outflow has only a role in stimulating synthesis and secretion of the saliva proteins in mammals. But our previous results from 1- and 2-DE analysis showed that the impairment of sympathetic innervation caused by reserpine promoted changes in protein profile of submandibular (SMG) and parotid glands (PG) and stimulation of adrenoceptors restored partially these effects. **Objectives:** Investigate the role of sympathetic outflow in the mouse submandibular and parotid glands by identification of proteins of the gland whose synthesis are regulated by this innervation. **Methods:** Adult Swiss male mice were divided into control (n=3), treated with reserpine (n=3) for 6 days (0.5mg/kg, ip) and treated with reserpine for 6 days (0.5mg/kg, ip) and phenylephrine plus isoprenaline (20mg/kg, ip) in the 6th day (n=3). Proteins from extracts of SMG and PG were analyzed by 2-DE. Only highest density specific spots from each group were digested with trypsin and their protein content were identified by ESI-LTQ XL/Orbitrap MS. Searches were made using NCBInr database. All information about proteins identified was collected on UniProt. **Results and Discussion:** In the SMG, reserpine treatment decreased the number of proteins related to cytoskeleton organization and protein biosynthesis, and increased the number of proteins related to metabolic and biosynthetic processes. In the PG, reserpine treatment reduced the number of proteins involved in cytoskeleton organization, transcription, intracellular signaling, and respiratory chain and increased the number of proteins involved in biosynthetic, metabolic and catabolic processes. The administration of adrenoceptor agonists to the reserpine-treated mice reverted these effects in the both glands. It is interesting to note that only in the PG of reserpine-treated animals, the number of secreted proteins was higher than in control and the administration of adrenoceptor agonists reverted this effect suggesting that sympathetic outflow is important to the process of exocytosis in this gland that has a high secretory rate under stimulated conditions. These results suggest that the sympathetic outflow has an important role for cellular homeostasis in mouse salivary glands, but acts in a different way in SMG and PG. The knowledge of the proteins that have their synthesis regulated by sympathetic outflow will bring new insights on the role of sympathetic outflow in different salivary glands and could open promising new avenues for treatments of oral diseases.

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3.09 Rat striatal tissue ^{3}H-GABA release: Characterization and effects of experimental parkinsonian injury

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Introduction: Parkinson's disease, a progressive and neurodegenerative condition, is related to the deaxth of neurons located in Substantia Nigra compacta, a component of Basal Ganglia. When nigral dopaminergic neurons die, this modulatory pathway is lost leading to imbalance between direct and indirect pathways, the latter having its activity increased over the former. Striatum has an essential role in receiving and filtering motor signals from cortex and thalamus and its major neuronal populations are composed by GABAergic neurons, showing how important is GABA in this modulation. Striatum receives dopaminergic projections from Substantia Nigra compacta and in its absence the typical signals and symptoms of the disease arise. **Objectives:** We aimed to characterize GABA relase at this structure, assessing the effect of other transmitters as well the role of some intracellular signaling molecules in this process. **Methods:** We employed the superfusion method and release of preloaded radiolabeled GABA from chopped striatal tissue. Nigral injury was produced by stereotaxic surgery and 6-OHDA microinjection at medial forebrain bundle (mfb). Several drugs were used to evaluate different steps in transmitter release. **Results and Discussion:** We found that the release is strongly calcium-dependent and follows vesicular exocytosis model; in addition, the striatal GABAergic subpopulation of neurons studied here undergo little influence of glutamatergic and cholinergic afferents. However, dopaminergic drugs complexly regulate striatal GABA release and it also shows high involvement of calmodulin. We wonder if some antipsychotic drugs that act over calmodulin owe their therapeutical effects, or at least part of it, to this activity and if in 6-OHDA unilateral lesion parkinsonism model there is communication between injuried and healthy hemispheres after the establishment of the injury and neuronal rearrangement process.

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3.10 Crotoxin inhibits capillary-like structures formation and endothelial cells integrin expression

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Introduction: Crotoxin (CTX), the major toxin from *Crotalus durissus terrificus* rattlesnake venom, presents antitumor activity *in vivo* and *in vitro*. Angiogenesis, the development of new blood vessels, is essential for tumor growth and metastasis. Integrins, the major family of cell surface molecules, facilitates bidirectional communication between endothelial cells (ECs) and matrix extracellular which may contribute in many processes such as cell proliferation, attachment and migration, thus, formation of capillary network, essential for tumor development. So, controlling tumor-associated angiogenesis may be a promising strategy for cancer therapy. **Objectives:** In the present work it was investigated the effect of CTX on capillary network formation on matrigel and EC integrin expression, using *in vitro* models. **Methods:** Pre-treatment: Murine endothelial cells line derived from thymus hemangioma (t.End.1) were treated at concentration with CTX (1.2μg/mL), for 1 h. *In vitro* angiogenesis assay: 50μL of matrigel (9.3mg/mL) were added to 96-well plates and polymerized for 1h at 37ºC. EC ($1.5x10^4$), pre-treated or not, were added to each well and incubated for 6 h. The formation of capillary networks was quantified by counting the total capillary length in 5 fields per well. For immunofluorescence, $5x10^4$ cells, pre-treated or not, were placed on coating of fibronectin (FN-3μg/mL), collagen I (COL I-10μg/mL) and laminin (LN-10μg/mL) over the coverslips. After overnight incubation in presence of culture medium, the coverslips were permeabilized and fixed. After 3 washes for 10 minutes, the coverslips were blocked using natural goat serum for 1 h at room temperature (RT). Then, ECs were incubated in the presence of primary antibodies anti-α_2 (coating of COL I and LN) and anti-α_v (coating of FN) incubated overnight at RT. After this period, the coverslips were washed and incubated with secondary antibody goat and rhodamine for a period of 1 h at RT and absence of light. Then, the coverslips were washed and assembled. Integrin expression was analyzed in confocal microscope. **Results and Discussion:** The results have shown that CTX inhibited the formation of capillary network (66%). CTX also decreased expression of α_2 and α_v integrins, as well protrusion formation and actin polymerization, thus cell migration was affected. In conclusion, we demonstrated that CTX inhibits *in vitro* angiogenesis through inhibition of α_v and α_2 integrins expression and capillary network formation, which maycontribute for the inhibitory effect of the toxin on tumor growth.

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3.11 Influence of environmental enrichment on anxiety and basal pain sensitivity in rats

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Introduction: Chronic pain is a public health serious problem, since many types of pains are still intractable. It has been found that environmental enrichment (EE) can alter the perception of nociceptive stimuli as well as the analgesic response induced by opioids, suggesting a relationship between well-being and analgesia. **Objectives:** The aim of this work was to evaluate the role of animal welfare in pain sensitivity of rats against different noxious stimuli and the response of these animals to different opioid drugs. **Methods:**Male Wistar rats (Butantan Institute) were used. All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 1050/2013). After weaning, animals were assigned to these conditions: enriched rats were housed in groups of five in the cages and given different novel objects every week on a regular basis. The control group did not receive objects. After 6 weeks under these conditions, the effects of EE on anxiety were analyzed using Plus Maze test, whereby it was counted how many times the animal entered in both opened and closed arms as well as the time spend in each arm. Moreover, the basal pain sensitivity to thermal stimulus was evaluated using the tail flick test, before and 1 h after morphine (2 mg/kg) or saline (control) treatments. **Results and Discussion:** The results showed that the EE had a positive effect on anxiety of rats. Enriched animals (n= 10) entered in opened arms and spent more time on them when compared to control group (n= 7). This result shows that the EE used in this work is effective, since is describe in the literature that many different types of EE can diminish the anxiety of experimental animals. In relation to thermal pain sensitivity, in normal conditions, it was observed no differences between enriched and non-enriched animals. On the other hand, morphine, an opioid agonist drug, in a submaximal dose, induced analgesic effect in the EE group that was not observed in control group. This suggests that EE enhances the effectiveness of opioid analgesic drugs. The data presented here is an indicative for the importance of EE on both limbic and analgesic system.

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3.12 A lipidomic approach to understanding the action of a snake venom phospholipase A_2 from in monocytes that leads to foam cell formation

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Introduction: Phospholipases A_2 (PLA_2s) are the largest group of lipid-modifying enzymes and display a wide range of functions in innate response triggering activation of diverse inflammatory cells. Viperidae snake venoms are rich sources of GIIA secreted PLA_2s ($sPLA_2$s) that present high grade of homology with inflammatory GIIA mammal $sPLA_2$s. **Objective:**To characterize: 1) the profile of released and metabolized fatty acids (FA) and 2) lipid droplet (LD)/foam cell formation in human monocytes stimulated by MT-III, a $sPLA_2$ from *B. asper* snake venom. **Methods:** Monocytes were isolated from peripheral blood of human donors (Ethic approval 14/2010 UV-Spain) and cultured in RPMI. Lipidomic analysis was assessed by Gas chromatography and HPLC coupled to mass spectrometry analysis to detect and quantify lipid species. LD biogenesis was evaluated by immunofluorescence assay followed by confocal microscopy analysis. qPCR was used to measure RNA levels of enzymes of FA metabolism. **Results and Discussion:** Data showed a marked decrease in the content of saturated and polyunsaturated FA (60%) from phospholipids (PLs) as well as of total arachidonic acid (AA)-containing PL, dependent on PL species and time of exposure, in MT-III-stimulated cells. Moreover, large amounts of membrane lyso-PL and free FA, such as oleic and AA were found in MT-III-stimulated monocytes as compared to control cells. MT-III induced appearance of high levels of triacylglycerol and cholesterol enriched in palmitoleic, stearic, and oleic acid (50-100%) along with an increase in LD and foam cell formation. LD formation was reduced by treatment of cells with the acyl CoA synthetase inhibitor triacsin C, suggesting the importance of reacylation process in this MT-III-induced effect. Moreover, the increased FA arising from the enzymatic hydrolysis of phospholipids correlated with activation of mitochondrial-oxidation pathway and promoted eicosanoid synthesis. Collectively, our data provide a complete lipid profile of the response of monocytes to a venom $sPLA_2$, reveal significant connections between free FA and LD biogenesis, leading to foam cell formation, and give new insights into the roles of human $sPLA_2$s in inflammatory lipid disorders, such as atherosclerosis.

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3.13 The antinociceptive effect of crotalphine results from a peripheral interaction between opioid and cannabinoid systems

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Introduction: Crotalphine, a 14 amino acid peptide, is a structural analogue of a novel analgesic peptide first identified and isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus*. This peptide induces a potent and long lasting (2-5 days) analgesic effect, when evaluated in experimental models of pain, being mediated by activation of peripheral *kappa* (acute pain) or *kappa* and *delta* (chronic pain) opioid receptors. Despite the opioid-like activity, studies indicate that the peptide does not directly bind to opioid receptors, suggesting that the release of endogenous opioids may be responsible for its analgesic activity. **Objectives:**The aim of this work was to evaluate the possible interaction of opioid and cannabinoid systems in the crotalphine antinociception.**Methods:** Male Wistar rats (160-180g, Butantan Institute) were used. All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 622/2009). Hyperalgesia was induced by prostaglandin E_2 (PGE_2, 100 ng/paw). The antinociceptive effect of crotalphine was determined using the paw pressure test. The activation of cannabinoid and opioid receptors was evaluated by imunoblotting assays using conformation-state sensitive antibodies.The contribution of endogenous opioids to crotalphine antinociception was investigated using antibodies anti-β-endorphin (5 µg/paw), anti-enkephalin (50 µg/paw) and anti-dynorphin A (1 µg/paw). The release of endogenous opioids (β-endorphin, met-enkephalin and dynorphin A) was confirmed in the paw tissue by enzyme immunoassays (Peninsula Laboratories). Binding evaluation in cannabinoid receptors (CB_1 or CB_2) was performed in a transfected cell line in a competitive assay.**Results and Discussion:** Theresults demonstrate that crotalphine increased the activation of CB_2 and *kappa* receptors in the tissue from rat paw. Antibody anti-dynorphin A inhibited the antinociceptive effect of crotalphine. *In vitro* enzyme immunoassay confirms that crotalphine induces the local release of dynorphin-A and this effect is blocked by CB_2 receptor antagonist. Crotalphine is able to direct binds to CB_2 receptors.These results indicate that the effect of crotalphine involves the release of dynorphin-A, the endogenous agonist of *kappa* opioid receptors, being this release dependent on the CB_2 receptors activation.

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3.14 Endothelial prostacyclin biosynthesis inducedby snake venom secretory phospholipase A_2: terminal synthase requirement

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Introduction: Prostacyclin (PGI_2) is a member of the prostaglandin family and the main lipid mediator released by endothelial cells (ECs). As with other prostaglandins, PGI_2 is produced in vascular ECs in response to physiological/pathological stimuli by sequential enzymatic reactions initiated by phospholipases A_2 (PLA_2). The main toxin of *Crotalus durissus terrificus* snake venom is a secretory groupIIA PLA_2 (CB) that exerts neurotoxic, myotoxic and anti-inflammatory effects. PLA_2 enzymes act on membrane phospholipids releasing AA, which is subsequently converted into prostanoids by an enzymatic cascade, with tissue-specific synthases as terminal components. Several reports have shown that prostacyclin terminal synthase (PGIS) is akey regulatory molecule of PGI_2 biosynthesis. **Objectives:**Therefore, in this study the effect of CB on PGIS protein expression profile in endothelial cells was examined. **Methods:** Rat primary microvascular ECs in culture were used (Butantan Institute Ethical Committee n. 76410). These cells were stimulated with CB (0.4 μM) or culture medium only (control) for selected time intervals (2, 4, 6, 12 and 24 hours) and then used for Western Blotting analysis. **Results and Discussion:** The incubation of ECs with non-toxic concentration of CB (0.4 μM) induced a significant increase (73,4%) of prostacyclin synthase protein expression only after 8 hours incubationin comparison with controls. In the same time-course, CB subunit up-regulated endothelial prostacyclin release, with a peak in the later incubation times (6, 8, 12 hours, with increases of 221%, 141%, 122%, respectively). These results indicate that up-regulation of PGIS protein levels in later periods of stimulation by CB should contribute to maintain prostacyclin biosynthesis induced by CB in ECs. These findings indicate a key regulatory requirement for thissnake venom group IIA PLA_2-induced biosynthesis of prostacyclin in endothelial cells.

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3.15 Aldehyde dehydrogenase 2 activation reduces neurophatic pain in rats

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Introduction: Neuropathic pain control remains a challenge and an unmet clinical need. Aldehyde-dehydrogenase 2 (ALDH2) is a mitochondrial enzyme responsible for the metabolism of reactive aldehydes. Aldehydes accumulation has been recently related to increased pain. Recent data from our group has been shown that activation of ALDH2, using a small molecule called Alda1, displays a potent antinoceptive effect in a model of carrageenan-induced hyperalgesia (intraplantar, i.pl) in rats. ALDH2 activation induces analgesia by reducing aldehydic load. However, the role of ALDH2 in neuropathic pain control is still unknown. **Objectives:** Therefore, we propose to investigate the involvement of ALDH2 in neuropathic pain, using Alda-1, an ALDH2 pharmacological agonist which selectively enhances the activity of ALDH2. **Methods:** The experiments were conducted in C57/BL mice following the protocols approved by the Butantan Institute Ethical Committee (976/12). Neuropathic pain was induced by sciatic nerve chronic constriction injury (CCI). The nociceptive threshold was determined, before and 14 days after surgery, using the electronic von Frey method. Fourteen days after surgery, a dose response curve for Alda-1 was performed (5, 10 and 20 mg/Kg, s.c. route) and the pain threshold evaluated. **Results and Discussion:** CCI decreased the pain threshold when compared to values obtained before surgery (67%). Alda1 (5 mg/Kg) decreased CCI-induced nociception at 1 and 2 hours after its administration (33 and 94%, respectively, compared to baseline). Alda1 (10 and 20 mg/Kg) also increased nociceptive threshold (88 and 91% at 1h, respectively, compared to baseline and 84 and 95% at 2 h, compared to baseline, respectively). No differences in pain threshold were detected 3 h after Alda1 injection. Alda1 vehicle treatment did not modify pain threshold. The results indicate that activation of ALDH2 by Alda1 reduces CCI-induced nociception. This effect was not dose-dependent and lasted 2 hours. Our data propose a novel mitochondrial target for neuropathic pain control. Therefore, Alda-1 may be a novel therapeutic drug class to reduce neuropathic pain.

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3.16 Conformational analysis and effect of crotalphine on peritoneal macrophages

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Introduction: Crotalphine (CRP) is a 14-mer peptide isolated from the venom of C. durissus terrificus "rattlesnake" that triggers long-lasting antinociception (3-5 days, p.o., i.v., s.c.),mediated by κ and/or δ-opioid receptorsactivation. Initial results obtained in our laboratory have also shown the involvement of cannabinoid receptors and the release of endogenous opioid peptides in this action. Immune cell-derived opioid peptides produced mainly by granulocytes and macrophages play a substantial role in the modulation of pain via interactions with opioid receptors located in peripheral sensory nerve fibers. **Objectives:** In this work, we analyzed the structure of *CRP* and studied the effect of the fluorescent analogue, CF-[Gln[1]]-*CRP*, on peritoneal macrophages. **Methods:** CD spectra of *CRP* and their analogues [Gln[1]]-*CRP*, CF-[Glu[1]]-*CRP* and CF-[Gln[1]]-*CRP* have shown band indicative of the presence of random and helix structure. PEP-FOLD software was used for modeling the structure of *CRP*. The best model was evaluated by PROCHECK software indicating that 100% of residues are in allowed regions. **Results and Discussion:**Fluorescent microscopy analysis of peritoneal resident or LPS-activated macrophages treated with CF-[Gln[1]]-*CRP* showed that this peptide internalizes and is distributed in the cytoplasm. LC/ESI-MS analysis of supernatant of LPS-activated peritoneal macrophages cultures treated with CF-[Gln[1]]-*CRP* have shown the presence of dynorphin A (1-13) and met-enkephalin. The results indicate that *CRP* structureis composed of random coil and helix similar to high sequence identities of the C-terminal regions of PLA_2 from *Trimeresurus stegneri* and *Agkistrodon halys pallas*. Data also suggest that macrophages could be the cellular target of *CRP*.

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3.17 Altered distribution of cell cycle phases in cultured astrocytes induced by Mlx-8 and Mlx-9 phospholipases isolated from *Micrurus lemniscatus* snake venom

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Introduction: Neurotoxins with characteristic phospholipase A2 activity, isolated from *Micrurus lemniscatus* snake venom, induce cell death in cultured neurons. Glial cells, in particular astrocytes, are partners at the synapses, being influenced by neurotransmitters and releasing gliotransmitters that modulate the synaptic transmission. Astrocytes, differently from neurons, are cells that retain the ability of proliferation. Flow cytometry analysis enables the identification and quantification of cells in the different phases of the cell cycle (G0/G1, S, G2/M phases). **Objectives:** The purpose of this work was to evaluate the effects of two phospholipases toxins, Mlx-8 and Mlx-9, isolated from *Micrurus lemniscatus* snake venom on the distribution of different cell cycle phases in cultured astrocytes. **Methods:** Astrocytes were obtained by trypsin (0,25%) digestion (10min at 37°C) of eight pineal glands isolated from adult male Wistar rats. The cells obtained were cultivated in 75 cm^2 culture flasks in DMEM medium with 10% fetal calf serum and 1% penicillin-streptomycin (37ºC, 5%CO_2, 16h). The supernatant was then removed and astrocytes remained attached to the surface of the flasks. After 2 weeks, astrocytes were transferred to 6-well plates (1,2 mL/well) and remained for 24h. Mlx-8 or Mlx-9 toxins (1, 10, 100 and 1000ng/mL) were incubated for 24h. DNA content was measured by flow cytometry to assess the distribution of the cell cycle phases. Cells were suspended in PBS and fixed by the addition of cold absolute ethanol to a final concentration of 70% and incubated for 1 h at 4° C. Cells were washed and suspended in PBS containing 1% fetal bovine serum. They were stained for 3 h with 50mg/mL propidium iodide and treated with 200 mg/mL RNase-A, in order to cleave single stranded RNA. Analysis was performed using FACS Calibur (Becton Dickinson) and the distribution of the cell cycle phases was determined with CellQuestPro and ModFit LT 3.0 softwares. **Results and Discussion:** In astrocytes, low doses of Mlx-8 caused cell cycle arrest in the G0/G1 phase (10 and 100 ng/mL) and S phase (1, 10 and 100 ng/mL) while cells treated with a higher concentration of MLX-8 (1000 ng/mL) were arrested in the G2/M phase. The toxin Mlx-9 (100 and 1000 ng/mL) induced also a cell cycle arrest at G2/M phase. DNA fragmentation was not changed after Mlx-8 or Mlx-9 toxin treatment. These findings suggest that the toxins from *Micrurus lemniscatus* could inhibit astrocytes cell growth and that this effect could be related to senescence-mediated induction of antiproliferative responses.

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3.18 Biosynthesis of PGE_2 induced by BaP1 metalloproteinase in synoviocytes: role of COXs, EP4 receptor and NF-kB pathways

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Introduction: Snake venom metalloproteinases (SVMPs) present homology with matrix metalloproteinases (MMPs), which are increased in inflamed joints during arthritis. We have previously demonstrated that BaP1 induces inflammation in rat joints with release of prostaglandin E_2 (PGE_2), major mediator of pain in arthritis. Studies on SVMPs may thus provide insights into the functions of MMPs. **Objectives:** To analyze the mechanisms of BaP1-induced PGE_2 release in B type synoviocytes (BSy), the main articular cell source of PGE_2, evaluating: a) release of PGE_2; b) role of COXs in PGE_2 release; c) expression of COX-1 and -2, mPGES-1, IκB-α and EP4 receptor (EP4R), and d) involvement of NF-κB and EP4 receptor in BaP1-induced effects. **Methods:** BSy were isolated from male Wistar rat knee joint (CEUAIB 576/09). Levels of PGE_2 were measured by EIA. Gene expression of COX-2 and mPGES-1 was determined by qPCR and protein expression of COXs, mPGES-1, IκB-α and EP4 receptor determined by W. blotting. Participation of COXs, NF-κB and EP4R on BaP1-induced effects was evaluated by specific pharmacological treatments. NF-κB translocation into nucleus was measured by EMSA. **Results and Discussion:** Stimulation of BSy with BaP1(12.5μg/mL) induced release of PGE_2 (1-6h), compared with control cells (RPMI alone). Treatment of BSy with the inhibitors of COX-1 and -2, valeril salicilate and NS398, respectively, reduced BaP1-induced PGE_2 release. BaP1 induced COX-2 gene (1h-3h) and protein (30min-3h) expression, and PGESm-1 gene and protein expression (3-6h) without modification on COX-1 expression. Inhibition of NF-kB by SN50 compound abolished BaP1-induced PGE_2 release and significantly decreased BaP1-induced COX-2, but not mPGES-1 protein expression. In agreement, BaP1 induced translocation of NF-κB and decreased protein levels of IκB-α. Pre-treatment of cells with AH23848, an EP4R antagonist, abolished PGE_2 production and protein expression of both COX-2 and mPGES-1. Moreover, EP4R protein expression was increased in BaP1-stimulated BSy. These data indicate that BaP1 is able to directly stimulate BSy for release of PGE_2 and expression of COX-2, mPGES-1 and EP4R. Activation of NF-κB pathway by BaP1 is relevant for expression of COX-2 and release of PGE_2. Moreover, engagement of EP4R by PGE_2 displays a positive feedback loop for expression of COX-2 and mPGES-1that amplifies biosynthesis of PGE_2 induced by BaP1. These findings suggest novel regulatory mechanisms for metalloproteinases in BSys.

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3.19 Aldehyde dehydrogenase-2 enzymatic activity regulates nociception in acute inflammatory pain models

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Introduction: Pain control remains a challenging unmet clinical need with few novel genetic or mitochondrial targets identified. Asians have a lower pain tolerance than Caucasians and African Americans, however, whether this difference has a behavioral or genetic origin is unknown. Additionally, whether an Aldehyde dehydrogenase 2 (ALDH2) mutation, which is present in 40% of East Asians, contributes to the lower pain tolerance in Asians is unclear. **Objectives:** To examine how ALDH2 may modulate the acute inflammatory pain response in wild type and genetically modified mice having the common ALDH2 mutation present in Asians, ALDH2*1/*2, and also, to determine whether a small molecule activator of the ALDH2 enzyme, Alda-1, could reduce pain in mice and rats. **Methods:** Male C57/BL6 mice (wild type and ALDH2*1/*2) and Wistar rats were used in a carrageenan-induced inflammatory pain model. Alda-1 or vehicle were injected subcutaneously. Von Frey filaments and the paw pressure method were used to assess the pain threshold in mice and rats, respectively. Molecular techniques to measure ALDH2 activity, aldehydic adducts, lipid peroxidation and acetaldehyde levels were also assessed. **Results and Discussion:** Carrageenan caused pain response in wild type mice when compared to baseline, which was exacerbated in ALDH2*1/*2 mutant mice (68±5% vs 25%±8% vs 92±7%#, respectively). The selective ALDH2 activator, Alda-1, reduced carrageenan-induced hyperalgesia in wild type mice (15±5%) and partially rescued the pain response for the ALDH2*1/*2 mutant, compared to the untreated mutant (58±6%). No differences in baseline pain scores were detected between wild type and mutant mice. In rats, carrageenan reduced ALDH activity (36 ± 5% compared to vehicle) and this reduction was rescued by Alda-1. Increases in 4-hydroxinonenal (4-HNE) adducts, malonyaldehydes (MDA) and acetaldehyde (ACth) levels occurred in carrageenan treated rat paws when compared to controls (158±36%, 77±32%and 50±5%, respectively). Alda-1 also significantly decreased 4-HNE, MDA and ACth levels (58±27%; 38±22%, 52±6%compared to controls). Our results indicate ALDH2 is essential for mediating inflammatory pain. The common genetic mutation examined in mice, representative of the ALDH2 mutation present in East Asians, could perhaps explain a genetic mechanism, rather than behavioral or cultural differences for the lower pain tolerance of Asians compared to other human races. Alda-1 may also represent a novel small molecule useful as an analgesic, regardless of race, for treating acute inflammatory pain.

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4. Immunology and Vaccines

4.01 Obtaining and isolation of Fc fragment of IgG from plasma of Magellanic penguins using affinity chromatography and papain digestion

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Introduction: The Magellanic penguins (*Spheniscus magellanicus)* are native of Argentina, Chile and Falkland Islands. These birds visit the Brazilian coast during their winter migration, and are sometimes taken into rehabilitation centers. In these organizations, however, the animals may be frequently diagnosed with infectious diseases as avian malaria, that can produce mass mortality in captive penguins. The immunological memory or active immune response to a parasite infection can be demonstrated by the production of distinct classes of antibodies or immunoglobulins. **Objectives:** This study aims to produce monoclonal antibodies against Fc region of IgG and IgM antibodies isolated from penguin plasma, which will be used for the development of serological test for avian malaria in penguins.**Methods:** The immunoglobulins were previously purified from plasma of healthy penguins through precipitation with caprylic acid associated to ammonium sulphate. The precipitated proteins were submitted to size exclusion chromatography to obtain the IgM and IgG fractions. The IgG antibodies were re-purified through affinity chromatography with CIM® r-Protein L Disk Monolithic Column, which binds to the Kappa light chain from immunoglobulins. The IgG-kappa was treated with immobilized papain (enzyme: substrate ratio of 1:30) for 4 hours and then once again submitted to the L-protein affinity chromatography and recovered from the flow-through. The efficiency of the enzymatic digestion and purification were analyzed by SDS-PAGE. **Results and Discussion:** The experiment of affinity chromatography with L-protein demonstrated the presence of IgG antibodies containing Kappa or Lambda light chains in the plasma of penguim. After papain treatment, the chromatographic profile of the IgG sample showed two peaks, the first that contains the proteins without light chain (Fc fragments) and the second containing the undigested IgG as well as the Fab fragment. The SDS-PAGE analyses under non-reductive conditions demonstrated a thick band between 50 and 75 kDa that is compatible with previous studies on the Fc fragment of chicken IgY. This result was confirmed by Western blotting using anti-chicken IgG antibody. These results combined indicate that the L-protein affinity chromatography is capable of separating penguin IgG antibodies based on the presence of Kappa light chain, as well as to separate the Fc and Fab fragments of penguin IgG following papain enzymatic digestion.

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4.02 The modulatory effect of high molecular weight components from *Ascaris suum* extract is dependent from DC-sign and MR receptors

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Introduction: Dendritic cells (DCs) when recognize the pathogens as their compounds through distinct receptors, as C type lectins receptors (CLRs), acquire the capacity to induce the specific cellular response. CLRs are involved in the homeostase of immune system as well as the recognition of carbohydrate structures. We previously described that high molecular weight components (PI) from *Ascaris suum* extract down-modulate the antigen-presenting cells activities. **Objectives:** Here, we analyzed the presence of glycosylated components in PI to suppress the DCs maturation and the binding to CLRs in DCs. In addition, was evaluated the role of C type lectins receptors of DCs incubated with PI to activate OVA-specific T cells. The down-modulatory effect of these glycan components obtained by affinity with ConA-Sepharose was analyzed in DCs incubated with LPS. **Methods:** The costimulatory molecules expression was studied in DCs incubated with LPS, LPS+PI, LPS+PI-ConA for 18 h. Purified DCs from OVA-primed mice were pulsed with OVA (200 µg/mL), OVA+PI (200+200 µg/mL), OVA+PI+mannan (200+200+50 µg/mL) or OVA+PI+α-CLRs (200+200+10 µg/mL) for 18 h. The PI binding to CLRs on DCs was analyzed by confocal microscopy. Immature DCs were incubated at 4°C with PI-Alexa488, BSA-Alexa488, PI-Alexa+mannan or PI-Alexa488+α-CLRs for 30 minutes and analyzed. **Results and Discussion:** The flow cytometry results showed that the ConA-linked components inhibited the expression of coestimulatory molecules on DCs induced by LPS compared with those observed on DCs incubated only with LPS.The T cell proliferative response was partially restored when DCs were pulsed with mannan or α-CLRs and incubated with OVA+PI. Higher fluorescence was observed in DCs incubated with PI-Alexa, in contrast, these PI-binding on DCs was inhibited when the cells were pre-incubated with mannan or α-CLRs. The results indicate the participation of the CLRs in the PI recognition by DCs and then triggering the modulation activity of these glycan structures.

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4.03 Action of the immunomodulator P-MAPA on the complement system and Toll Like Receptors in a model of inflammation induced by LPS

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Introduction: P-MAPA, a proteinaceous aggregate of ammonium and magnesium phospholinoleate-palmitoleate anhydride derived from *Aspergillus oryzae*, has been described as a promising immunomodulatory compound to be used as adjuvant therapy for infectious diseases and cancer.**Objectives:** On the present study, we analyzed the potential modulatory properties of P-MAPA on the complement system and TLR2 and TLR4, using an *in vitro* model of human whole blood. **Methods:** After approval of the ethical committee, blood from healthy donors (n=4) was collected with lepirudin and preincubated for 5 minutes at room temperature with PBS, P-MAPA or LPS (100 µg/mL). P-MAPA, LPS or PBS was then additionally added to the samples, which were incubated for 30 minutes, at 37 °C. After incubation, aliquots of blood were collected for analysis of CD11b, CD14, C3aR, C5aR, TLR2 and TLR4 expression in leucocytes, by flow cytometry. Plasma was collected with EDTA for C3a, C5a, TNF-α, IL-6 and IL-8 measurements. P-MAPA was also incubated with normal human serum for testing complement activation. **Results and Discussion:** P-MAPA (500 µg/mL) was able to reduce the lytic activity of the classical (20.3±0.3%) and alternative (26.1 ± 2.9%) pathways, as determined in haemolytic assays, but not of the lectin pathway, as determined by ELISA. P-MAPA (125, 250, 500 or 1000 µg/mL) alone increased the expression of CD11b (3714±44.5; 4213±57.3; 546±258.8; 7270±417.9; Median of Fluorescence Intensity ± SEM) and decrease of C5aR (632.0±15.6; 545.0±5.6; 509.0±19.8; 473.5±16.3), in a dose dependent manner, and had no effect on C3aR, TLR2 and TLR4 expression, compared to PBS (CD11b: 1739±207.9; C5aR: 580.0±21.2) Only P-MAPA at 1000 µg/mL increased CD14 expression (523.0±16.9; PBS = 398.5±33.2). Combination of LPS with P-MAPA decreased the expression of all receptors, compared to PBS. P-MAPA (125, 250, 500 µg/mL) alone promoted high production of C3a (18127±971.5; 45299±1619; 47608±80.9) and C5a (110.1±4.4; 223.0±2.0; 208.0±1.7) compared to PBS (C3a = 3720±26.9 ng/mL; C5a = 76.2±9.3 ng/mL) and in combination with LPS, generation of both anaphylatoxins increased. P-MAPA (500, 1000 µg/mL) also induced the production of cytokines TNF-α and IL-8, but not IL-6. Therefore, our data indicates that P-MAPA has proinflammatory properties and its use as immunomodulator for LPS-associated infectious diseases still requires further analysis.

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4.04SBA-15 silica adjuvant enhances the immuneresponse and modulates sensibility to bacterial toxins

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Introduction: Ordered nanopores particles formed of silicon oxide (SBA-15) are promising adjuvant vectors. **Objectives:** Here we explored how silica might act in the enhancement of phagocytosis, the recruitment of inflammatory cells and also in the modulation of toxicity after administration of exo or endo toxins encapsulated in SBA-15. **Methods:** Confocal microscopy and flow cytometry tests confirmed that the pre-incubation of FITC labelled ovalbumin with SBA-15 improved the phagocytosis of the protein by bone marrow derived DC from BALB/c mice. The recruitment of cells evaluated by flow cytometry after 24 and 72 hours of subcutaneous injection of SBA-15 showed the presence of inflammatory cells at the site of injection, especially mature DC. BALB/c mice immunized intraperitoneally (ip) with diphtheria toxin (1 µg/mL): SBA-15 (25 µg/mL) presented 100% of survival rate whereas all mice that received diphtheria toxin (1 µg/mL): Alum (25 µg/mL) died. However, BALB/c mice inoculated ip with LPS from *Salmonella* Typhimurium(100 µg/mL) in SBA-15 (1000 µg/mL) showed a higher death percentage (55%) compared to the LPS (100 µg/mL): Alum (1000 µg/mL) group (36%).**Results andDiscussion:** Though, both experimental groups were able to produce similar specific antibody titres indicating that SBA-15 worked well as immunological adjuvant, reducing the toxicity of the diphtheria toxin in immunizations but increasing the death of mice injected with LPS. These data confirm that SBA-15 acts as adjuvant for antibody responses and suggest that its effects may reflect enhanced availability of antigen without modifying its properties.

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4.05 Thermal stability of IgG and F(ab')$_2$ horse anti-venoms

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Introduction: According to the World Health Organization, venomous animals accidents are a public health problem, mainly in rural areas of tropical and subtropical countries. The anti-venom therapy is the only treatment capable of neutralizing the envenomation systemic effects. The heterologous anti-venoms are composed of intact immunoglobulins (IgG) or their fragments (F(ab) or F(ab')$_2$, obtained from the plasma of immunized animals, usually horses. Anti-venoms should be kept between 4ºC and 8ºC, but the storage conditions are not always ideal, which could affect the anti-venoms stability and efficiency. **Objectives:** To evaluate the stability of IgG and F(ab')$_2$ horse anti-venoms, subjected to different temperatures and incubation periods. **Methods:** Anti-Arachnid, Anti-Bothropic, Anti-Bothropic-Crotalic, Anti-Bothropic-Lachetic, Anti-Bothropic-Crotalic-Lachetic, Anti-Crotalic, Anti-Scorpionic and Anti-Lonomic anti-venoms, produced between 2007 and 2010, were provided by Butantan, Vital Brazil and Clodomiro Picado Institutes. Anti-venoms aliquots were incubated for 7 and 30 days at room temperature (25°C) or incubator (37°C). After the incubation period, the specific antibody titers were determined by ELISA and compared to the titers of samples kept at 4°C. In the case of anti-venoms produced in Brazil, the titers were determined against the specific reference venom(s) for which they were produced. In the case of anti-venoms from Costa Rica, venoms from the same genus were used. Unrelated anti-Botulinic serum was used as negative control. **Results and Discussion:** For some samples, titers remained stable at the temperatures and times analyzed, while others showed a decrease in the titer, which may suggest a reduction in antibodies binding capacity. Surprisingly, it was observed an increase of titers for some samples. It is suggested that this phenomenon may be due to technical artifact through the formation of protein aggregates in samples incubated at 25°C and 37°C, which would lead to an increase in the optical density of reactions. This hypothesis corroborates other published studies. Further investigations are being performed in order to confirm the formation of protein aggregates in these samples. Our results suggest that the anti-venoms may not be stable at the temperatures and periods analyzed, at least concerning the formation of protein aggregates, which may contribute to the development of early adverse reactions observed in patients submitted to serum therapy.

Supported by: FAPESP, CNPq, INCTTOX

4.06 Cytokines and chemokines produced in the early periods after inflammation stimulus modulate the rapid neutrophil progenitors expansion in bone marrow: a mechanism related to high acute inflammatory reactivity

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Introduction: Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response (AIR) exhibit significant differences in the average number of migrant neutrophils and in the protein content of the inflammatory exudate in response to polyacrylamide beads (Biogel). As a result of an inflammatory stimulus, the hematopoietic system accelerates the expansion of myeloid progenitors by increasing the production of granulocytes, a phenomenon called emergency myelopoiesis. One of the major factors of the higher inflammatory capacity of AIRmax mice, which can reach 20-fold difference related to AIRmin mice, is the larger production of neutrophils by the bone marrow (BM). **Objectives:** In the present study we set out to evaluate the cellular and molecular factors that confer hematopoietic potential difference between AIRmax and AIRmin mice and their association with the intensity of AIR. **Methods:** For this purpose, the animals were assessed at steady-state and at different time periods (1.5, 3, 6, 12 and 24 h) after stimulation with Biogel P-100®. We evaluated: 1) the BM cellularity by total leukocyte count; 2) cytokine production in the serum exudate detected by magnetic microspheres; 3) the expansion of myeloid progenitors by flow cytometry assay; 4) the *in vitro* BM proliferation; 5) gene and protein expression of hematopoietic cytokine receptors by real-time PCR (qPCR) and flow cytometry, respectively and 6) the expression of transcription factors by qPCR. **Results and Discussion:** Our results show that only AIRmax mice presented the ability to develop an emergency myelopoiesis induced by Biogel. These results were confirmed by the significant increase of total leukocytes, local G-CSF, GM-CSF and chemokines (KC, MIP-2) production in the first 3 h of AIR induction and also expansion of common myeloid progenitors (CMP) followed by BM granulocytes differentiation at various stages of development. On the other hand, in AIRmin mice, any of these phenotypes were altered in the first 3 h when compared to steady-state conditions. Thus, we conclude that the ability of the AIRmax mice to develop a higher AIR, compared to AIRmin mice, is associated to the myeloid progenitor cells expansion, which in turn might be determined by the local soluble factors produced during the first hours of inflammation.

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4.07 How *M. leprae* Hsp65 influences the immune response in genetically selected aged mice?

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Introduction: The immunosenescence process affects both humoral and cellular responses leading to an imbalance of the individual homeostasis. Heat shock proteins may trigger innate immune responses and are involved in immunosenescence and autoimmunity. Previous data showed that the Hsp65 of *M. leprae* interfere with the mean survival time in female H_{III} mice, not affecting males. **Objectives:** To characterize selected cellular and humoral alterations after intraperitoneal administration of *M. leprae* Hsp65 in genetically selected mice for High (H_{III}) or Low (L_{III}) antibody production (9-months-old) and in its F_1 hybrids. **Methods:** Mice were injected through the intraperitoneal route with 2.5ug of *M. leprae* Hsp65 and cellular or humoral alterations were analyzed by flow cytometry or ELISA, respectively. **Results and Discussion:** aged H_{III} female injected with Hsp65 presented a survival decrease of 42% when compared to untreated group (control); no changes in IgG1 or IgG2a anti-Hsp production were observed in H_{III} and L_{III} mice. Regarding the cellular changes, aged H_{III} female Hsp65-group presented amplified frequency in $CD4^+CD154^+CD28^+$ cells ($p<0.01$) and reduced percentage of B and activated $CD11c^+$ cells ($p<0.01$) in the spleen, and increased percentage of $CD11c^+$ and $NKG1A/C/E^+$ cells ($p<0.01$) in the blood compared to control. Hsp65 acts like an imbalance trigger: post-injection, the aged F_1H female Hsp65-group died 2 months after the first death, as observed in aged H_{III} females; however, there was no statistically significance compared with F_1H control group. Furthermore, aged F_1H and F_1L female showed amplified frequency of naïve T cells and CD11c cells in spleen ($p<0.001$). Our results confirm the sex dichotomy and the sex effect of the Hsp65 interference in the immunity of aged mice, becoming evident in females. Next, we will characterize innate immune cells in peritoneal cavity after Hsp65 inoculation; in addition, the role of myeloid-derived suppressor cells will be investigated as these cells increase during ageing process and have been associated with attenuation of experimental autoimmune diseases.

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4.08 Effect of the association of ordered mesoporous silica SBA-15 to LPS in the modulation of toxicity and antibody production of mice selected for maximal acute inflamatory response (AIRmax)

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Introduction: The mesoporous silica SBA-15 presents advantageous structural properties that allow the association with molecules, low toxicity, and good chemical, thermal and hydrothermal stability. It has shown adjuvant effect in association with various antigens, inducing high production of antibodies. It also was able to lower the toxicity of diphteric toxin in the immunization of horses. Mice selected for maximal acute inflammatory response (AIRmax) are genetically heterogeneous and are susceptible to endotoxic shock induced by LPS. **Objectives**: Our aim is to evaluate the association of ordered mesoporous silica SBA-15 to LPS in the toxicity and antibody production of AIRmax mice. **Methods**: The toxicity of LPS free or associated with SBA15 was evaluated by the determination of DL_{50}. Groups of AIRmax mice received 50 µg, 75 µg or 100 µg of *Salmonella Typhimurium* LPS, adsorbed or not in SBA15, by intraperitoneal route and dead was registered up to 72h after inoculum. In order to evaluate the antibody production, groups of AIRmax mice received by ip route LPS free or adsorbed in SBA15 or $Al(OH)_3$ for comparison, with the dose of 10% DL50. These groups were compared with BALB/c mice receiving the same antigens. Blood samples were collected 10 and 30 days after inoculum and IgM and IgG anti-LPS antibodies were determined by ELISA. **Results and Discussion:** The DL_{50} values were: LPS= 139.65 µg; LPS+Sílica= 96.28 µg; LPS+$Al(OH)_3$= 118.71 µg, showing that the association with SBA15 increased the toxicity effect of the endotoxin. Both mice lineages produced IgG and IgM anti-LPS antibodies. In the AIRmax mice there were no significant differences in antibody levels between the groups receiving LPS + SBA15 and LPS free, showing that no adjuvant effect was caused by silica by the intraperitoneal route. Animals receiving LPS + Al(OH)3 exhibited great individual variation in antibody levels, nevertheless there was no statistically significant difference between the groups. The great individual variations in the antibody response of AIRmax mice may be due to the genetic heterogeneity of this lineage. The BALB/c mice, genetically homogeneous, showed significantly higher IgG antibody levels compared with AIRmax, with less individual variation. This difference was not observed in the IgM anti-LPS antibodies. Further experiments are being designed to clarify the influence of the association of SBA-15 in the increase of the LPS toxicity as well as in the antibody production.

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4.09 Acute inflammation loci influence tissue repair in mice

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Introduction: AIRmax (high inflammation) and AIRmin (low inflammation) mouse lines homozygous for *Slc11a1 S* alleles, produced by genotype-assisted mating, differ in ability to completely repair ear holes. AIRmax^{SS}mice showed fast ear tissue regeneration while AIRmin^{SS}mice did not show regeneration after ear punch. **Objective:** To characterize the inflammatory reaction in AIRmin^{SS} and AIRmax^{SS}mice after ear punch. **Methods:** Two-millimeter ear holes were done in these mice for the characterization of inflammatory reaction. **Results and Discussion:** Thelocal inflammatory response was more intense in AIRmin^{SS}than AIRmax^{SS}mice 24 and 48 h after ear punch, which was demonstrated by histomorphometric analysis, multiplex assay for inflammatory cytokines such as IL-1β, IL-6 and MIP-2 and higher levels of MPO. Global gene expression analysis demonstrateddistinct over-representedbiological themes between AIRmax^{SS} and AIRmin^{SS} control mice. At 24 h after punch, both AIRmax^{SS} and AIRmin^{SS} showed significant up-regulated genes related to inflammation. However, angiogenesis, epidermis development and collagen catabolic process were expressed only in AIRmin^{SS}. All down-regulated genes in response to wounding in AIRmax^{SS} were represented to muscle contraction which is known to be involved in healing with scarring. Microarray results were validated by qPCR. These results suggest that the degree of inflammatory response in the early events after injury drives tissue regeneration or wound healing after ear punch.

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4.10 The effect of the *Slc11a1* gene on pristane-induced arthritis chronic phase

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Introduction: Genetic and environmental factors contribute to the development and establishment of rheumatoid arthritis. The *Solute carrier family 11a member 1* (*Slc11a1*) gene is involved in the ion transport at the endosomes in macrophages and neutrophils, interfering in their activation. Mice homozygous for *Slc11a1S* allele (AIRmax^{SS}) selected from the high inflammatory response AIRmax line are more susceptible than AIRmax^{RR} to pristane-induced arthritis (PIA). **Objective:** The aim of this study was to investigate the effect of *Slc11a1* gene polymorphism in the activation of peritoneal macrophages during pristane-induced arthritis chronic phase. **Methods:** In order to studythe chronic phase of PIA, AIRmax^{RR} and AIRmax^{SS} mice received two i.p. injections of 0.5 mL pristane at a 60-day interval, and arthritis development was assessed for 180 days.**Results and Discussion:** Multiplex analysis of serum and peritoneal macrophage culture supernatants showed higher production of IL1β, IL-6, TNFα, IL-10 and MIP-2 by AIRmax^{SS}. We also identified increased H_2O_2 and NO release by AIRMax^{SS} macrophages. Significant differences ($p<0.001$) were found in expression, by AIRMax^{SS} macrophages, of metalloproteinase genes (*Mmp-2* and *Mmp-9)* and their inhibitors (*Timp-1* and *Timp-3)*. Histological analysis of paws of AIRMax^{SS} mice after PIA showed a moderate inflammatory process, characterized by mononuclear cells. These results suggest that *Slc11a1* alleles modulate cytokines profile and proteases contributing for arthritis severity.

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4.11 Gene expression profile of peritoneal macrophages from AIRmax mice bearing *Slc11a1R* and *S* alleles during arthritis

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Introduction: Macrophages play a central role in the pathogenesis of rheumatoid arthritis and *Slc11a1* gene regulates macrophage and neutrophil activity. Mice homozygous for *Slc11a1S* allele (AIRmax^{SS}) selected from high inflammatory response AIRmax line are more susceptible than AIRmax^{RR} to pristane-induced arthritis (PIA), suggesting that *Slc11a1* or other closed-linked gene interacts with inflammatory *loci* to modulate this experimental arthritis. **Objectives:** The aim of this work was to identify candidate molecules involved in the development and progression of PIA. **Methods**: AIRmax^{RR} and AIRmax^{SS} mice received two ip injections of 0.5 mL pristane on days 0 and 60; PIA incidence and severity of PIA were assessed for 180 days. PCR arrays were used to examine the gene expression profile of peritoneal macrophages during the chronic phase of PIA, in order to identify candidate molecules that may be involved in the development and progression of the disease. **Results and Discussion:** Eighteen genes of the chemokine/receptors network had higher constitutive expression in AIRmax^{SS} than AIRmax^{RR} macrophages, which may favor arthritis susceptibility. Upregulation ($p<0.05$) of 7 and 12 genes were observed in the macrophages of pristane-treated AIRmax^{SS} and AIRmax^{RR} mice, respectively. Histological analysis of paws from arthritic AIRMax^{SS} mice showed a moderate inflammatory process, characterized by mononuclear cells. Kidneys were infiltrated with mononuclear cells and presented glomerular basement membrane thickening and hyaline cylinders in renal tubules in both lines. These results reveal that *Slc11a1* alleles modulate the expression profile of chemokine/receptor genes, which may be involved in the increased susceptibility of AIRmax^{SS} mice to PIA.

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4.12 Can Humoral immunity enhance development of skin tumors?

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Introduction: The incidence of tumor may be associated with interactions between innate and adaptive immune responses. Evidences suggest that antibodies can generate inflammatory conditions facilitating malignant progression in a mouse model of skin cancer. Mice genetically selected for High (H) and Low (L) antibody production treated with 7, 12-dimethylbenzanthracene (DMBA) were used to study the association between humoral immunity and tumor susceptibility. **Objectives:** Verify the influence of genetic factors relevant to antibody production on skin tumor development. **Methods:** Skin tumor was induced at the shaved back of mice by epicutaneous application of DMBA (50µg in 0,1mL acetone) for 5 consecutive days, and controls were treated with acetone. IL-1β, IL-6, Cxcl-2, IL-10, TNF-α were measured by Milliplex in skin homogenate after 48 hours of treatment and serum TGF- β was analyzed by ELISA. **Results and Discussion:** DMBA treatment led H and L mice to an intense initial vascularization in male mice and around 15 days all mice presented a superficial cutaneous inflammation. This lesions regressed in about 30 – 60 days in all mice and skin papillomas started to be detectable in H mice (76%, n = 30) and L mice (14%, n = 29). Skin tumor multiplicity and malignancy increased with time and was significantly higher in H than in L mice. Higher levels of IL-10 were observed in H mice treated with DMBA than L mice. On the other hand L mice produced more IL-6. Treated mice produced more IL-1β, Cxcl2 and TNF-α than their controls.Large amounts of TGF-β were detected in High mice after 120 days. In this phase TGF-β could be associated with tumor progression in inflammatory microenvironments. The High responder mice were more susceptible than Low responder mice showing the effect of the genetic selection for humoral response on skin tumor susceptibility and predisposition to lung tumorigenesis.

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4.13 Phenotypically selected mice as a model of susceptibility and resistance to intestinal Ischemia/ Reperfusion injury

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Introduction: Intestinal Ischemia/reperfusion (IR) injury produces a systemic inflammatory state, which can lead to severe organ dysfunction. The physiopatological mechanisms arising from that process have been studied in a variety of experimental models. **Objective:** The aim of this study was to evaluate the regulatory mechanisms of local and systemic inflammation after intestinal IR in two lines of mice phenotypically selected for maximal (AIRmax) or minimal (AIRmin) local Acute Inflammatory Response to polyacrylamide beads. **Methods:** Mice were subjected to 45 min of superior mesenteric artery occlusion followed by different periods of reperfusion. Control groups were sham operated and normal mice. The local (gut) and systemic (lung) inflammatory reactions due to IR were evaluated by Myeloperoxidase (MPO) activity and gene/protein expression. Intravital microscopic (IM) was performed in mesenteric venules and the gut bacterial translocation (BT) was measured in mesenteric lymph nodes (MLN) and spleen. **Results and Discussion:** We observed by IM that the cell adhesion levels in I/R AIRmax mice were significantly higher ($p<0.001$) than other groups at 1 h of reperfusion with consequent high intestinal cellular infiltration measured by MPO activity. Moreover, the local expression of Icam1, TNFα and Vhl mRNA were also higher in I/R AIRmax line ($p<0.05$). The systemic effects of the I/R were more pronounced in AIRmax mice. The BT to the MLN in I/R AIRmax mice was 3-fold higher than I/R AIRmin. The lung inflammatory reaction measured by MPO activity showed a progressive increase up to 4 h. Higher *Hif1a*, *Vhl* and *Il6* gene expression was detected in the lung parenchyma of AIRmax mice. The proteomic analysis revealed a variety of proteins differently expressed in lung from AIRmax and AIRmin mice such as Profilin-1, Tropomyosin beta chain, Keratin type II cytoskeletal 1, S100A9, Anexin1, Eukariyotic Translation Factor 5, Rho GDP- dissociation inhibitor 2. All these proteins were involved in adhesion, migration, or apoptosis. Our results show that AIRmax mice are more sensitive than AIRmin mice to intestinal I/R injury with intense local/systemic inflammatory reaction, differential related gene/proteins expression and bacterial translocation. This interline difference is according to selection phenotypes indicating these lines as an appropriated model for the study of I/R regulation concerning inflammatory mechanisms.

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4.14 Effect of high fat diet on inflammatory response induced by Biogel in mice selected for maximal and minimal acute inflammatory response

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Introduction: Adipose tissue has been increasingly recognized not only as storage of lipids but as an organ involved in systemic inflammatory responses and insulin resistance. Elevated levels of cells and cytokines associated to the innate and adaptive immune response are observed in adipose tissue. **Objectives**: In this study, mice phenotypically selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response were used to investigate the interaction between inflammation, obesity and glucose levels. To this purpose, we investigated the effect of a high fat diet (HFD) on the inflammatory response induced by polyacrylamide beads (Biogel) in an air pouch model. **Methods**: Groups of AIRmax and AIRmin mice were submitted to HFD for ten weeks. Food intake was monitored daily and body weight monthly. For measurement of glucose levels, animals were injected with glucose (10 μg, i.p.) and blood samples were collected 30, 60 90 and 120 minutes later. The number of leucocytes was determined 24 h after the Biogel injection. Total protein content of the exudates was determined by spectrophotometer at 280 nm. **Results and Discussion**: Obese Male AIRmax presented significant higher levels of glucose 30, 60 and 90 min after glucose injection compared to lean AIRmax and obese AIRmin. In addition, obese AIRmax animals presented a decreased number of leukocytes in the Biogel pouch in comparison to lean controls. However, no significant differences in cell number were observed in AIRmin animals regardless of the diet. The total content of protein in the exudates was diminished in obese AIRmax mice in comparison to lean AIRmax and both groups of AIRmin. These results suggest that obesity interfere with the acute inflammatory response that characterizes the AIRmax strain of mice. Whereas, the same effect is not observed in AIRmin mice that are constitutively low inflammatory responders.

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4.15Crotoxina from *Crotalus durissus terrificus* modulates the macrophage and lymphocyte populations in acute intestinal inflammation in mice

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Introduction: Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBDs) possible due to an abnormal activation of the immune response against constituents of the luminal flora. Infiltrated lymphocytes and macrophages have a key role in exacerbation of pathogenesis on inflammatory bowel disease. Crohn's disease is classically regarded as a Th1 mediated inflammatory disorder in lamina propria. Recently, it was verified that Th17 cells are also involved in this inflammatory intestinal response. Macrophages, including 'classical' (M1) and 'alternatively' activated (M2) have been studied as a target for the treatment of inflammatory bowel diseases such as Crohn's disease. Crotoxin (CTX) is the main component of the *Crotalus durissusterrificus* rattlesnake venom and it has immune suppressive effect. **Objective:** Here, we evaluated the modulatory effect of CTX on the murine model of colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS). **Methods:** Male BALB/c mice (n=20) were anesthetized and 0.1 mL of TNBS in 45% ethanol was administered by intrarretal route to induce acute colitis. The control mice received only 0.1 mL of 45% ethanol. After 18 h, the mice received the CTX or PBS ip and 4 days after induction they were sacrificed. Entire colon was quickly removed and gently cleared of feces for measurement of myeloperoxidase (MPO)enzymeactivity and cytokines secretion by ELISA. Macrophages and lymphocytes were isolated from lamina propria for flow cytometry analysis. **Results and Discussion:** Clinical and histological scores showed that the CTX-administration decreased the disease progression in TNBS-colitis induced mice. MPO activity was significantly higher in the TNBS-group than in TNBS group that received CTX which indicates a reduction of the index of tissue inflammation by the toxin administration. High IL-17, IL-6, IL-1, IFN-γ and TNF-α secretion was verified in cell supernatants from TNBS-mice. In contrast, these cytokines secretion was lower in mice that received the CTX. Higher percentages of $CD11b^+CD68^+$ (M1), $CD4^+ROR\gamma^+$ and $CD4^+Tbet^+$ cells were observed in lamina propria of TNBS-colitis mice, however when CTX was administered in TNBS-group these cell populations were decreased as observed in ethanol control groups. Our preliminary results suggest a modulatory role of CTX in the macrophages and lymphocytes differentiation in acute intestinal inflammation induced by TNBS in mice.

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4.16 Characterization of a novel recombinant monoclonal antibody, scFv, against BaP1, a P-I metalloproteinase from *Bothrops asper* snake venom

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Introduction: The BaP1 toxin is a P-I class of snake venom metalloproteinase relevant in the local tissue damage associated with envenomations by *Bothrops asper*. We constructed a recombinant single chain fragment variable (scFv)monoclonal antibody against BaP1 (scFvBaP1). It contains VH and VL domains linked by a flexible $(G_4S)_3$ polypeptide.**Objective:**To evaluate capacity of scFvBaP1 to recognize and neutralize important actions of BaP1. **Methods:**scFvBaP1 was cloned into pMST3 vector in fusion with SUMO protein. Citoplasmic expression of this construction was sucessfully active in C43 (DE3) bacteria. The ability of monoclonal antibody (MaBaP1) and the scFvBaP1 to recognize total venom from *Bothrops asper* and BaP1 was assessed by ELISA. The ability of scFvBaP1 to neutralize BaP1-induced gastrocnemius muscle necrosis was estimated by incubating with 35 µg or 20 µg of BaP1, respectively, with scFvBaP1 (10:1 molar ratio). The capacity of scFvBaP1 to neutralize BaP1-induced local inflammatory reaction in peritoneal cavity was estimated by incubating of BaP1 with scFvBaP1 (10:1 molar ratio). **Results and Discussion:**ELISA showed that scFvBaP1, as well as MaBaP1, was able to recognizeBaP1 as well as whole venom, while SUMO did not. ScFvBaP1 completely neutralized muscle necrosis induced by BaP1. Finally, BaP1-induced inflammatory cell influx and peroxide hydrogen production in peritoneal cavity was inhibited by scFvBaP1. Our data showed that scFvBaP1 specifically recognized and neutralized biological effects of BaP1, indicating the potentialuseof this recombinant antibodyfortherapy ofpoisoningcaused by this snake.

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4.17 Mast cell activation induced by MT-I, a Phospholipase A_2 isolated from *B. asper* Snake Venom: Role of enzyme activity and pathways involved

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Introduction: Mast cells are central elements of innate immune response. Upon stimulation these cells release a vast array of inflammatory mediators through degranulation and *de novo* biosynthesis. Snake venom phospholipases A_2 have been shown to induce *in vitro* MCs degranulation. However, the mechanisms involved in this effect and contribution of enzyme activity are still unknown. **Objectives:** This study aimed to evaluate the ability of MT-I, isolated from *Bothrops asper* snake venom, to induce MCs degranulation and PGD_2 release. Moreover, involvement of kinases (p38MAPK, PI3K and ERK1/2), intracellular phospholipases (calcium-independent and cytosolic PLA_2, PLC and PLD) and contribution of MT-I catalytic activity in MC degranulation was evaluated. **Methods:** RBL-2H3 mast cell lineage was used. MC degranulation was determined by measuring β-hexosaminidase release and PGD_2 was evaluated by Enzyme Immune Assay.Involvement of kinases and phospholipases in MT-I-induced MCs degranulation was evaluated by pharmacological interferences. Inhibition of catalytic activity was determined by incubation with p-bromophenacylbromide (0.1 mM). **Results and Discussion:** Incubation ofMCs withnon-cytotoxic concentration of MT-I (0.35 μM) resulted in increased MC degranulation by 64%, 102% and 95% at 30 min, 1 and 2 h, respectively, in comparison with control (3.96±0.53%, 3.72±0.3% and 3.60±0.24% respectively, n=6). In addition, MT-I (0.35 μM) induced a significant increase of PGD_2 released from MCs after 3 to 6 h incubation (n=3). Inhibition of MT-I catalytic site decreased MC degranulation (n=6). Pre-treatment of cells with PD98059 or SB202190, inhibitors of ERK1/2 and p38MAPK, respectively, did not affect MT-I-induced MC degranulation. Pre-treatment with wortmannin, inhibitor of PI3K, significantly reduced MT-I-induced MC degranulation by 53.7% at 30 min as compared with controls (n=6). Inhibition of $iPLA_2$ by Bel compound did not modify degranulation induced by MT-I, but inhibition of $cPLA_2$, PLC or PLD by treating cells with compounds Pyr-2 or U-73122 or FIPI, respectively, reduced MT-I-induced MC degranulation (29%, 25.2% and 25.4%, respectively, n=6). MT-I is able to induce a rapid and sustained MC degranulation and PGD_2 release from these cells. MT-I-induced MC degranulation is dependent on PI3K, $iPLA_2$, PLC and PLD, but not ERK1/2, p38MAPK and $iPLA_2$. Furthermore, the PLA_2 catalytic activity is relevant for MC degranulation.

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4.18 Bacterial endotoxindetection sensitivityof *Pseudomonasaeruginosa*in water forinjectionsamplesby"Gel Clot" andKineticsTurbidimetric techniques

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Introduction: Bacterial endotoxinisa complexlipopolysaccharide(LPS), integralcell wallofGram-negative bacteria that consists of threedistinct regions: hydrophilic, acidic andhydrophobic (lipid A,biological activity). The clinical effectsofLPSaretoxic action, leukopenia andfever. *Pseudomonas aeruginosa* isa Gramnegative, aerobic,bacilliform andcapable offorming biofilm. There aretwo types of techniquestodetect or toquantifyendotoxin using LAL test (*Limulus*AmoebocyteLysate)with differentsensitivities: gelclot - GC (semi-quantitative) andphotometric, kinetic turbidimetric - KT (quantitative). **Objectives:** This study evaluated the detection sensitivity of *P. aeruginosa* bacterial endotoxin in water for injection samples, by the GC and KT techniques. **Methods**: *P. aeruginosa* suspensions were prepared to contain a viable number of microorganisms of 155,11 and 1 CFU (colony forming unit) and were tested simultaneously in GC and KT. In GC a 100 μL of each suspension was added to the LAL reagent with detection sensitivity of 0.125 EU/mL, in triplicate, and incubated at 37 °C for one hour and observed the gel formation or not. In addition, the compatibility of the suspensions was performed with LAL reagent, by carrying out a positive control with endotoxin from *Escherichia coli*. In the KT, a standard curve of endotoxin was prepared from *E. coli*, to give a linear interpolation which allows the determination of endotoxin concentration in each suspension test. The procedure included the incubation of endotoxin standard and control solutions with the LAL reagent to obtain a calibration curve. **Results and Discussion:** The results of *P. aeruginosa* viable number found were higher than 0.125 EU/mL (GC) and concentrations of 1.14 and 0.10 EU/mL (KT), for 155 and 11 CFU respectively. The viable number of 1 CFU presented in GC less than 0.125 EU/mL and in the KT smaller than 0.05 EU/mL value. The validation criteria were included in both tests; it is compatible and the spike recovery showed values between 50 and 200%. With these results, we conclude that using the LAL reagent with 0.125 EU/mL sensitivity it is possible to detect *P. aeruginosa* bacterial endotoxin of viable number between 155-11 CFU dispensing the quantification in KT techniques. Viable microbial concentration of 1 CFU is not detectable in both techniques.

Supported by: Instituto Butantan

4.19 Bacterial endotoxin detection by turbidimetric kinetic method in hyperimmune sera

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Introduction: There is an urgent need of *in vitro* assays for *in vivo* method replacement in quality control of biological. According to national recommendations, pyrogen detection is performed through *in vivo* assay in rabbits. For the bacterial endotoxin detection, the LAL (limulus amebocyte lisate from *Limulus polyphenus*) reactive is used. There are two available techniques with different sensibilities: gel clot (semi-quantitative method) and fotometric method which includes turbidimetric and chromogenic techniques. **Objectives:** the aim of this study is to evaluate the possibility of the endotoxin detection in sera by the turbidimetric kinetic method. **Methods:** two batches of hyperimmune sera (anti-tetanic an anti-escorpionic) previously tested by in vivo test for pyrogens. In this technique 3 rabbits are used per batch. The animals received the product by their marginal ear vein and their temperature was monitored for 3 hours. By the end of the test, the temperature raise was evaluated. Any animal can show more than 0.5°C of temperature raise. These batches were also tested by turbidimetric kinetic method at 1:10 and 1:100 dilutions in three consecutive days. The in vitro method is performed by comparison to a calibration curve of standard *E. coli* endotoxin. **Results and Discussion:** the *in vivo* test revealed the presence of bacterial endotoxin in anti-tetanic serum and the absence in the anti-escorpionic serum. From the in vitro assays the anti-tetanic serum had endotoxin at 29.45 EU/mL (dilution 1:10; spike -27%), 30.86 EU/mL (dilution 1:10; spike - 105%) and 23.29 EU/mL (dilution 1:10; spike - 112%) and from the dilution 1:100, 15.82 EU/mL (spike – 101%), 21.47 EU/mL (160%) and 17.17 EU/mL (spike 113%). Preliminary results indicate the possibility of using the "in vitro" kinetic turbidimetric method for the bacterial endotoxins detection in hyperimmune sera with proven compatibility when used in 1:10 or 1:100 dilutions. These are promising results and further experiments are needed for reduction, refinement and replacement of the animals use and induce the search for alternative methods to replace “in vivo” tests.

Supported by: Fundação Butantan

4.20 Descriptive analysis of adverse events after immunization temporally associated to the immunobiologicals produced by the Instituto Butantan, Brazil, 2012-2013

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Introduction: Although immunization has succeeded in reducing the global burden of disease and death, a number of concerns converge in confidence about the safety of vaccines. When that trust is broken, hesitation can lead to delays and denials in addition to stop immunization programs and research, and may sometimes cause disease recurrence and the occurrence of outbreaks and epidemics in susceptible groups of the population. Therefore, monitoring of adverse events following immunization (AEFI) is essential to assess the benefit-risk and ensure the success of immunization programs. The Instituto Butantan (IB) implemented the Pharmacovigilance area in late 2009 and in these four years has been improving the ability to detect cases and/or signs of AEFI related to biopharmaceuticals by it. **Objectives**: Evaluate the safety of biological products of IB in reported AEFI cases occurring in Brazil in 2012 and 2013. **Methods**: Descriptive analysis using data from AEFI reported to the Pharmacovigilance of IB. **Results and Discussion**: There were 729 reported AEFI related to 249 individuals. Of these, 54.2% were reported spontaneously, 18.1% were requested, 17.3% were collected in the regular media and 7.6% were identified through literature review. A total of 96 (38.6%) AEFI were classified as serious. Regarding the suspect products, 80% of the cases were related to influenza vaccine and 4% (10) were related to the anti-venoms serum produced by IB. Importantly, over 50% of reported cases had no information about the manufacturing laboratory. The major events occurring among the reported cases were: vaccine failure (16.1%), followed by vaccination error (12.0%), fever (10.0%), malaise (8.0%), local pain (8.0%) and cough (7.6%). We also emphasize the occurrence of 15 (6.0%) product quality complains. The data show that there were no relevant events associated with a product manufactured by IB. However, there is a need for enhance the surveillance of AEFI in order to have better information, which will allow a better understanding of the profile of the most frequent adverse events and the benefit-risk of our products.

Supported by: Fundação Butantan

4.21 Characterization of *Corynebacterium diphtheria* vaccinal strain by multi locus sequence typing

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Introduction: *Corynebacterium diphtheriae* is a pathogenic bacterium that causes diphtheria. The incidence of this disease reduced drastically due to the implementation of the National Immunization Program (NIP) of the Ministry of Health.with the introduction of Diphtheria Toxoid, one component of the combined vaccines (DTP, dT, DT), in national campaigns. Butantan Institute produces Diphtheria Toxoid by growing toxigenic *Corynebacterium diphtheriae* Park Williams 8 strain in industrial scale. Recently WHO published recommendations for production and quality control of diphtheria vaccines that suggests a molecular strains characterization, including study such as Multi Locus Sequence Typing (MLST), Pulsed fildgel electrophoresis (PFGE) or restriction fragment length polymorphism (RFLP). The Multilocus Sequence Typing (MLST) has been introduced as an approach for studying the molecular epidemiology of bacterial pathogens and the sequence data are truly comparable between laboratories that is an important advantage over the other typing methods, such as RFLP, randomly amplified polymorphic DNA and PFGE, furthermore, MLST is more amenable to quantitative analyses, allowing the establishment of quantitative genetic relationships between clinical isolates. **Objectives:** The aim of this study was to characterize the *Corynebacterium diphtheria* vaccinal strain by MLST.**Methods:***C. diphtheriae* PW8 strain was cultivated in Loefller medium during 24 hours. The DNA extraction was done using Cell and tissues-Roche Kit. The amplification of the ***atpA*, *dnaE*,** *dnaK*, *fusA*, *leuA*, *odhA* e *rpoB*genes was performed as described by Jolleyet al. 2004. The PCR products were purified from the agarose gels and the sequencing was performed in ABI 3130XL. The sequences were analyzed in CLC genomics and compared in the *Corynebacterium* MLST Database. **Results and Discussion:** The extraction of DNA yield 127.87 ng/μl with A260/280 1.72. The amplification products generate lengths from *atpA* 378 pb, dnaE 354 pb, dnaK 345pb, fusA 360pb, leuA 384pb e odhA 381pbe rpoB 410 pb. The analysis of DNA sequencing characterized *C. diphtheria*vaccinal strain (PW8) in atpA (14), dnaE (2), dnaK (23), fusA (4), leuA (2), odhA (14) e rpoB (2). This characterization of the vaccinal strain will allow the molecular monitoring in Diphteria Toxoid production during the next years, according to WHO recommendations.

Supported by: Fundação Butantan

4.22 Optimization process of Diphtheria Toxoid production

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Introduction: Diphtheria is a contagious disease spread by direct physical contact or breathing that aerosolizedsecretions of infected individuals with *Corynebacterium diphtheriae*. Butantan Institute produces diphtheria toxin by growing toxigenic *Corynebacterium diphtheriae* Park Williams 8 strain in industrial bioreactor (500 L). After cultivation the diphtheria toxin is separated, concentrated, detoxified by formaldehyde and purified to obtain Diphtheria Toxoid that is used in associated vaccines such as DTP (Diphtheria, Tetanus and Pertussis), DTP plus *Haemophilus influenzae* type b, DT (Diphtheria - Tetanus children use), dT (Diphtheria - Tetanus adult use) as well to immunize horses for anti-sera production. The production process started with cultivation of *C. diphtheriae* working seed in solid medium and then to liquid IB medium in order to obtain the inoculum for fermentation process. The inoculums preparation is a very important step to get an optimum diphtheria toxin titer in large scale. **Objectives:** Evaluate the diphtheria toxin production and bacteria growth in industrial scale, using different methods for the inoculums preparation: bioreactor "Wave" (GE) and the traditional technology inshaker. **Methods:** The inoculums were prepared using "Wave" bioreactor with horizontal agitation and a flask in a shaker with a rotatory agitation, both with the same volume of IB culture medium at 36ºC during 24 h. After that, were inoculated into bioreactor containing IB medium (250 L). During the fermentation, samples were taken to measure the Optical Density (O.D.) in a 590 nm, flocculation test (Lf) and SDS PAGE. **Results and Discussion:** The O.D. average after 64±2 hours of fermentation using as inoculums "Wave" bioreactor was 27.9% above the usual fermentation. The results of flocculation limit in the conventional method after 48 and 64 hours of fermentation and concentrate diphtheria toxin were 80 Lf/mL, 110 Lf/mL and 470 Lf/mL respectively. The average titers obtained using Inoculum prepared with"Wave" was 155 Lf/mL, 170 Lf/mL and 720 Lf/mL in 48 and 64 hours of fermentation and the concentrate diphtheria toxin, respectively. The SDS PAGE showed a molecular weight around 58 KDa that correspond to the Diphtheria toxin with visible high concentration toxin in "Wave" methodology. The "Wave" methodology uses disposable bags, representing a great advantage since it requires no sterilization and cleaning validation, and mainly the result indicates that the "Wave" technology allow to increase above 50% of the diphtheria toxin production.

Supported by: Fundação Butantan

4.23 MPLA as adjuvante anti-Leptospira vaccine

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Introduction: Leptospirosis is caused by spirochete *Leptospira*, the serological classification is based in the lipopolysaccharide (LPS), the major outer membrane component in *Leptospira interrogans*. LPS hydrolyzed resulting immunophosphoryl lipid A (MPLA), a fraction that is recognized by receptors on the host immune system cells and acts as adjuvant when present in vaccine formulations. The protein LigA is a vaccine candidate shown to induce protective immunity against challenge with virulent bacteria in animal model, although this antigen cannot avoid persisting kidney colonization. **Objective**: This work aims the purification of LPS from *Salmonella* and *Leptospira* and MPLA obtaining, quantification and characterization for studies as vaccine adjuvant. LigA and OmpA were chosen to be tested with the different MPLA as adjuvant. **Methods**: The hot phenol method was used for LPS extraction, and analyzed by silver stained Urea-SDS-PAGE. LPS was hydrolyzed to MPLA and was analyzed by TLC and mass spectrometry. Quantification of MPLA was measured by phosphate.The MPLA from *Salmonella* and from *Bordetella pertussis* plus alhydrogel were tested as adjuvant with the Leptospira antigens LigA and OmpA, in hamster and then challenged with virulent Leptospira interrogans Copenhageni. Kidneys of surviving animals were analyzed about the presence of Leptospira by bacteria isolation, PCR and immune detection on imprinted kidney. **Results and Discussion**: The LPS from Salmonella is extracted in aqueous phase of hot phenol, while LPS from *Leptospira* was separated in the phenol phase, indicating a more lipophilic molecule. The LPS were analyzed by SDS-PAGE showing the expected profile of LPS from *Salmonella* as a ladder of bands, while LPS from *Leptospira* showed discrete bands. The mild acid hydrolysis of *Salmonella* LPS was analyzed by TLC and mass spectrometry showed that lipid A molecule was being recovered, indicating that stronger acid hydrolysis was needed to remove the proximal phosphates. All LigA immunized animals survived after challenge with virulent Leptospira corroborating data from the literature. MPLA from Salmonella also resulted protective, and the significance of increased protection is being measured. Possible colonization of the kidneys by the bacteria is being analyzed. The procedure for obtaining MPLA from *Salmonella* was established and the purified molecule showed the same profile as the commercial MPLA when analyzed by mass spectrometry and TLC. The animal assay showed interesting results, and immune response by antibodies titers is still being measured as well as kidney colonization.

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4.24 Immunogenic potential of Leptospiral flagellins in the development of a subunit vaccine against leptospirosis

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Introduction: Leptospirosis is a zoonosis of global importance caused by pathogenic leptospires that colonize the renal tubules of wild and domestic animals. Leptospiral whole-cell vaccines are being used, but only to promote protection against the serovar in the preparation and failing to induce short-term immunity. The Leptospira C-terminal portion of immunoglobulin like protein A (LigAC) was able to induce immune protection against leptospirosis. However, immunization with LigAC did not confer sterilizing immunity. Flagellins have been considered a promising adjuvant for vaccine development. Leptospires have two periplasmic flagella that are formed by two classes of proteins (FlaA and FlaB), only FlaB proteins show homology with important regions that elicit TLR5-dependent responses. **Objectives:** In the present study, we have evaluated the ability to induce the TLR5 activity and the adjuvant activity of five *L. interrogans* sorovar Copenhageni flagellins (FlaB1, FlaB2, FlaB3, FlaB4 and FlaB5) in the protective immunity of LigAC against lethal challenge in hamsters. **Methods:** The recombinant 6xHis-tagged flagellins expressed in *E.coli* were purified by nickel affinity chromatography. Hamsters were immunized subcutaneously with purified flagellins with LigAC as well as in combination with alum or a cocktail of five flagelins. **Results and Discussion:** Experimental data showed that all flagellins could activate the TLR-5 receptor. In challenge assays, hamsters immunized with FlaB2 and LigAC demonstrated protection against death and a significant reduction in the colonization of kidneys. Control animals vaccinated with PBS died with symptoms of leptospirosis, and hamsters vaccinated with commercial vaccine survived after challenge. ELISA demonstrated that the most immunogenic protein was FlaB4, and western blotting assays showed that only FlaB2 was recognized by sera from infected hamsters, sera from hamsters immunized with the commercial vaccine and with recombinant flagellin *pool*. Taken together, the data of this study suggest a new formulation for the development of a subunit vaccine for leptospirosis.

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4.25 Virulence factor polymorphism in pertussis vaccine strain

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Introduction: Recently several countries reported an increased incidence of pertussis. One possible explanation for this resurgence is the expansion of strains antigenically distinct from those in the vaccines. Variation in the genes *prnA*, *ptxA-E*, *fhaB*, *fim2*, and *fim3* and other surface-associated proteins (tcf, brkA, vag8 and ompQ) has been reported in clinical isolates from the Netherlands, Finland, Italy, Japan, Poland and the USA. **Objectives:** This study was designed to compare the virulence factors and promoters of the Brazilian vaccine strain (Bp137) with Tohama strain, 18323 strain used in the potency test and Chinese strain (CS) used to prepare the Chinese aP vaccine. **Methods:** The whole genome sequencing of Bp137 was performed on 454 GS Jr and MiSeq platforms. The reads were assembled with Tohama genome as reference and analysis of 18 genes related with the virulence of the bacteria and the promoter region of *ptx* were done using the CLC Genomics Workbench. **Results and Discussion:** The *prn* alignment shows a substitution of THR_{532} (Tohama and CS) for ARG_{532} (Bp137 and 18323) and 18323 strain presents 7 more amino acid substitutions and a missing sequence of 9 nucleotides. PtxA differences consists on a GLU_{68} (Bp137 and 18323) while ASP_{68} in the others; VAL_{232} in Bp137 while ILE_{232} in Tohama, CS and MET_{232} in 18323. In PtxB, the Bp137 and 18323 strains have a SER_{45} however the Tohama and CS have a GLY_{45}. The *ptx* promoter (*ptxP*) alignment shows SNP in Bp137 identical to 18323 in contrast to the others. FhaB present a GLN_{831} and an insertion of a Lysil-proline (K_{3482} P_{3483}) in Tohama and CS while a HIS_{831} in Bp137 and 18323. FhaS presents GLY_{34} in Bp137 strain and ALA_{34} in the others. Fimbria 2 (Fim2) shows LYS_{174} in Bp137 and ARG_{174} in the other strains. 18323 tracheal colonization factor (TcfA) displays at position 151, a stretch of 25 amino acids that is missing in the other strains and GLU_{52} instead of LYS_{52}. Adenylate cyclase toxin gene (*cyaA*) shows MET_{892} in Bp137 strain while VAL_{892} in the other strains. There were no amino acid differences in in PtxC, PtxD, PtxE, Fim3, FimA, OmpQ, Vag8, BrkA, FimX, FhaC and FhaL. The polymorphism Bp137 is characterized by PrnA(7), PtxA(4), PtxC(1), PtxP(2), Fim2(2), Fim3(1), TcfA(2), OmpQ(2), Vag8(2) alleles, distinct from alleles that have been described for several vaccine strains. This important information would help to understand the immune response against *B. pertussis* vaccination and may also explain the immune evasion mechanisms of this pathogen.

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4.26 Draft Genome of *Bordetella pertussis*Vaccinal Strain

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Introduction: *Bordetella pertussis* is a human pathogen which causes pertussis, a contagious respiratory disease. WHO estimates that in 2008 about 16 million cases of pertussis occurred worldwide, killing 195,000 children. In 2011, global official numbers of reported cases was 162,047. Vaccination is the most effective intervention to prevent pertussis. Resurgence of pertussis has been recently observed in some countries with high vaccination coverage. The Brazilian National Immunization Program (BNIP) uses the whole cell pertussis vaccines produced at Instituto Butantan. Despite the reduction in incidence of whooping cough after the introduction of vaccination, the risk of outbreaks remains a public health concern. **Objective:** We reported here the draft genome sequence of Butantan´s *B. pertussis* strain used in pertussis vaccine production to compose DTwP vaccine (Diphteria, Tetanus and Pertussis) for the BNIP. **Methods:** The sequencing was performed combining shotgun sequencing in 454 GS JR and on MiSeq-illumina and analyzed CLC Genomics Workbench 3.6.5. **Results and Discussion:** A total of 297,811 reads comprising 128Mbases with 32-fold coverage of the genome was generated using two shotgun sequencing in 454 GS JR system. The MiSeq sequencing generated 270,011 reads (39Mbases). A total of 829,782 reads were assembled in 288 contigs, the longest contig had an 84,859pb, the total clustered contig was 4,7Mbases and an average GC content 67.65%. When the reads were assembled with Tohama genome as reference 1,170,111 reads were matched and 93,970 were not aligned. The draft genome of Butantan´s *B. pertussis* strain contains 3439 protein-coding sequences, 51 tRNA and 9 rRNA genes. Compared with the published genome of Tohama I strain, 30 genes were exclusively present in Butantan´s strain, one region contained 20 genes that could be found in published genome of*B. pertussis* CS, the Chinese vaccine strain, but not in Tohama strain. In contrast, 27 genes present in Tohama strain were absent in Butantan´s strain. These differences were mainly related with transcriptional regulatory and metabolic systems. Some small insertion deletion events (indels) and 214SNPs were identified but need experimental confirmation. In conclusion, the genome sequence of a vaccinal *B. pertussis* strain used since the 80´s in the BNIP will provide the basis to understand the immune response against the whole cell pertussis vaccine as well as the molecular mechanisms in possible outbreaks due to the emergence of new pertussis strains.

Supported by: Fapesp, CNPq and FundaçãoButantan

4.27 Multilocus sequence typing of the Brazilian Pertussis Vaccine strain

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Introduction: *Bordetella pertussis* is a human pathogen that is the causative agent of pertussis (whooping cough), a very contagious respiratory disease. The most effective intervention to prevent pertussis is through vaccination. The Instituto Butantan is the only national producer of pertussis vaccine used to compose the DTP distributed throughout Brazilian territory. Recently WHO published a recommendation for the vaccines production that suggests a molecular strains characterization, including study such as Multi Locus Sequence Typing (MLST), Pulsed fildgel electrophoresis (PFGE) or restriction fragment length polymorphism (RFLP). The Multilocus Sequence Typing (MLST) has been introduced as an approach for studying the molecular epidemiology of bacterial pathogens and the sequence data are truly comparable between laboratories that is an important advantage over the other typing methods, such as RFLP, randomly amplified polymorphic DNA and PFGE. Furthermore, MLST is more amenable to quantitative analyses, allowing the establishment of quantitative genetic relationships between clinical isolates. **Objectives:** The aim of this study was characterize the Brazilian vaccinal pertussis strain (Bp 137) by MLST. **Methods:** The Bp 137 strain was cultivated in Bordet & Gengou during 72 hours. The DNA extraction was done using Cell and tissues-Roche Kit. The amplification of the *adk*, *fumC*, *glyA*, *icd*, *pepA* e *tyrB* genes was performed as described by Jolley & Maiden 2010. The PCR products were purified from the agarose gels and the sequencing was performed in ABI 3130XL. The sequences were analyzed in CLC genomics and compared in the Bordetella MLST Database. **Results and Discussion:** The extraction of DNA yielded 1629 µg with A260/280 1.83. The amplification products generated lengths from adk 426pb, fumC 402 pb, tyrB 421 pb, glyA 393 pb, pepA 418 pb and icd 411 pb. The analysis of DNA sequencing characterized the Brasilian vaccinal pertussis strain (BP 137) in adk (1), fumC (1), glyA (1), icd (1), pepA (1), tyrB (1). This characterization of the vaccinal strain will allow the molecular monitoring during the next years of pertussis production following WHO recommendations.

Supported by: Fundação Butantan

4.28 Evaluation of Vaccine Pertussis Low

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Introduction: The association of adverse events in immunization with Whole cell Pertussis Vaccine (wP) has led to the development of Pertussis Acellular Vaccine (aP). However, it is known that immunization with aP does not have the same efficiency as compared to immunization with wP vaccine. Adverse reactions of wP vaccine are attributed to the presence of lipopolysaccharides (LPS) major constituents of the outer membrane of Gram-negative bacteria. Butantan Institute developed a vaccine Pertussis Low (Plow) with a reduced amount of LPS, which is less reatogenic than traditional cellular pertussis vaccine. Its production cost is lower than the P vaccine and has the same immunogenicity of traditional whole cell vaccine. **Objectives:** The aim of this study was to scale up the pLow, comparing the Industrial pLow with pLow pilot production and traditional wP already produced by Butantan Institute. **Methods:** Two industrial methods of pLow production were evaluated: tangential flow filtration (TFF) and tangential filtration with organic solvent washing (TFFW). These methodologies were compared with pLow pilot produced by centrifugation and traditional wP. Endotoxic activity was measured by the LAL test (Limulus Amebocyte Lysate), the removal of the LPS was performed by KDO assay and the major antigens of *B. pertussis* as PT, FHA, Fimbriae and Pertactin was compared by Western Blot. **Results and Discussion:** There were no significant differences observed in the content of the major antigens in all vaccines tested by western Blot. The KDO assay showed 25%, 50% and 80% LPS reduction in vaccines produced respectively with TFF, TFFW and centrifuged methods compared with conventional vaccine. In LAL centrifuged vaccine had the best result with 80% less endotoxic activity. These results indicate that Pertussis Vaccine Low produced by centrifugation presented the best result with higher LPS removal and reduced endotoxic activity.

Supported by: Fundação Butantan

4.29 Spray dryer process for industrial scale up of *Bordetella pertussis* monophosphoryl lipid A production

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Introduction: Adjuvants help antigen to elicit an early, high and long-lasting immune response with less antigen, enabling to reduce costs of vaccine production. Butantan Institute developed a whole cell pertussis vaccine with lower reactogenicity and allowing LPS recovery as byproduct which, following acid hydrolysis, leads to production of *Bordetella*-derived monophosphoryl lipid A (MPLA), a potent adjuvant. This adjuvant production must be scale up in order to use in a vaccination program. Spray drying is commonly used to produce solid particles from liquid solutions and may reduce substantially the volume to be processed. **Objectives:** The aim of this study was to evaluate spray dryer methodology to scale up of MPLA production through mass spectral. **Methods:** LPS obtained from the previously detoxified whole cell pertussis vaccine by organic extraction. The LPS extracted was processed by spray dryer in different temperatures 40°C, 60°C and 80°C and compared with no dry LPS and dried by lyophilization process. All of them were submitted to acid hydrolysis with acetic acid for 90 min at 100ºC, followed by neutralization, acid evaporation to generate the final MPLA products and they were evaluated by direct infusion MS/MS mass spectral analysis, negatively mode using Bruker Esquire 3000 Plus Mass Spectrometer. **Result and Discussion:** The direct infusion MS/MS spectra MPLA obtained from LPS extracted by spray dryer in 40ºC displayed major peak at 1826.7 m/z. Other peaks of 1016.3, 1236.1, 1407.1 and 1568.3 m/z were observed. MPLA obtained from LPS extracted by spray dryer in 60ºC displayed a major peak at 1205.3 m/z. Other peaks of 1091.4, 1367.9, 1615.1, 1830.4 m/z were observed. MPLA obtained from LPS extracted by spray dryer in 80ºC displayed a major peak at 1205.2 m/z. Others peaks of 1079.2, 1330.3, 1536.1, 1774.7 m/z were observed. The MPLA obtained from no dry LPS displayed a major peak at 1205.3 m/z. Other peaks of 1482.2, 1755.5, 1997.0 m/z were observed. The MPLA obtained from LPS by lyophilization process a major peak at 1205.3 m/z. Other peaks of 1113.3, 1341.1, 1634.7, 1747.1, 1857.1 and 1983.3 m/z were observed. The major peak at 1205.3 m/z observed suggesting that it should be predominantly in hexa-acylated form, with breakdown products. The next step will be to evaluate these products *in vivo* to know if the differences observed in the mass spectrum impact in the adjuvant properties.

Supported by: Fundação Butantan

4.30 Evaluation of the Immune Response induced against the Pneumococcal Vaccine Candidate PotD

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Introduction: *Streptococcus pneumoniae* is a gram positive encapsulated human pathogen that colonizes the nasopharynx and causes pneumonia, bacteremia and meningitis. The current pneumococcal vaccines are based on capsular polysaccharides conjugated or not to carriage proteins, but these induce protection only against the included serotypes. In order to increase the cross-reactive protection, several pneumococcal proteins have been investigated as vaccine candidates. PotD belongs to the PotABCD complex; this is an important complex that works in the transport of the extracellular polyamines to the intracellular environment. PotD is a surface exposed, membrane-associated protein, being antigenically conserved in diverse capsular serotypes. **Objectives:** The aim of this study was to evaluate the immunological response induced against PotD in immunized mice. **Methods:** The gene fragment of potD was amplified by PCR and inserted into the pET-28a expression vector. The recombinant PotD was expressed in *E. coli* and purified by affinity chromatography. BALB/c mice were immunized with 3 doses of PotD at 15 days intervals. The antibodies production and their ability to bind onto the pneumococcal surface were evaluated by ELISA and Flow Cytometry, respectively. Also, we tested the antibodies ability to promote the opsonophagocytosis and killing of pneumococci bearing capsular polysaccharide or not. Furthermore, immunized animals were challenged i.p. and the peritoneal cells recovered were cultured and used to measure the H_2O_2 and nitric oxide production. **Results and Discussion:** rPotD was expressed and purified. Mice immunization with rPotD was able to promote high levels of IgG. The binding assay results suggest that the polysaccharide capsule interferes in the antibody binding to PotD expressed on the pneumococcal surface. Using anti-PotD antibodies, we observed that the opsonophagocitosis and killing of capsulated pneumococci was reduced when compared with an uncapsulated pneumococcal strain, and these results were in accordance with the antibody binding assay. Finally, challenged mice showed that rPotD immunization was able to increase the H_2O_2 production and induced significant amounts of NO. These results suggest that rPotD is a promising candidate to be included in a protein based vaccine, but requires a better understanding of its protection mechanism. The induction of a cellular immune response and protection of PotD-immunized mice from pneumococcal challenge will be investigated. In addition a hybrid protein containing PotD fused to PdT, a pneumolysin detoxified form with adjuvant properties has been constructed and will be investigated.

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4.31 Development of a purification method for pneumococcal surface protein A from clade 4 (PspA4)

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Introduction: The use of pneumococcal proteins as carriers in conjugate vaccines could be an alternative to reduce the production costs and increase coverage. Pneumococcal surface protein A (PspA) has shown promising results in animal essays and is conserved among pneumococcal strains, therefore an excellent vaccine candidate. **Objectives:** To develop a purification process for PspA4 in which the protein reaches a relative purity greater than 95% for use in a pneumococcal conjugate vaccine. **Methods:** Recombinant PspA4 was produced in *E. coli* (BL21 DE3) with the plasmid pET37+. 100g of cell pellet was suspended in 1.0 L of 10 mM NaH_2PO_4+2.5 mM EDTA buffer with 1% Triton X-100 and 1 mM PMSF. Cell disruption was done at 500 bar for 10 min by a continuous high pressure homogenizer, thus giving the Homogenate fraction. 0.2% cationic detergent CTAB was added to Homogenate, which was then centrifuged. The resulting supernatant, named Clarified, was applied to anionic chromatography in Q-Sepharose, and PspA4 was eluted with 300 mM NaCl, thus the pH was adjusted to 4.0. The fraction was frozen/thawed (crioprecipitation) and centrifuged. The supernatant was applied to cationic chromatography in SP-Sepharose, and PspA4 elution was achieved with 650 mM NaCl. The pH was adjusted to 6.5 and the sample was loaded to a Mixed Mode resin, CAPTO MMC, where PspA4 was recovered in the flow-through. The purity of PspA4 was determined by densitometry of SDS-PAGE bands and protein concentration by Lowry. **Results and Discussion:** Cell rupture was completed after 8 min, as no further decrease in cell density was observed by OD 600 nM after this time. The relative purity of PspA4 was 51% in the Homogenate and it was increased to 62% in the Clarified fraction, with no loss of PspA4. Anionic chromatography increased PspA4 purity to 75% with a recovery of 60%. Crioprecipitation further purified the protein, reaching 90% of relative purity with recovery of 59%. After cationic chromatography, PspA4 purity reached 94% with a recovery of 61%. Finally, after mixed mode chromatography, PspA4 purity reached 98% with recovery of 79%. Among purification steps, Clarification with CTAB, anionic chromatography and crioprecipitation showed the highest purification factors (1.2 each). Although the two last steps showed both purification factors of 1.04, they were necessary to achieve purity >95%. As a whole, the process increased PspA4 purity from 51% in Homogenate to 98% after Capto MMC (overall purification factor: 2.4). PspA4pro total recovery after the last chromatographic step was of 17%. Although the protein was obtained with a purity level above the required, the process still needs refining in order to improve the global yield.

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4.32 Development of protein vaccines against *Streptococcus pneumoniae*: characterization of the adjuvant components of the cellular pertussis vaccine

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Introduction:*Streptococcus pneumoniae* is the leading cause of bacterial pneumonia worldwide. Pneumococcal diseases cause 1 million deaths of children under five years annually, mainly in developing countries. PspA has been shown to be an important antigen for the development of a protein vaccine against *S. pneumoniae*. We have previously shown that the whole cell pertussis vaccine produced at Instituto Butantan (wP) is an effective adjuvant to PspA, stimulating the production of antibodies against the protein and leading to protection of mice against a respiratory lethal challenge with *S. pneumoniae*. **Objectives:** Here we propose to evaluate the molecular basis of *Bordetella pertussis* (Bp) adjuvant properties through the combination of different Bp mutants or purified components ofthe bacteria with the PspA5 (PspA from clade 5) antigen **Methods:** BALB/c mice were immunized once through the nasal route with PspA5 alone or in combination with wP or inactivated Bp mutants (BPLOW and BPRA) or the parental strain (BPSM). Twenty one days after the challenge, sera were collected and the animals were challenged with the ATCC6303 pneumococcal strain. Survival was observed for 10 days. Anti-PspA5 antibody concentrations in the sera were measured by ELISA and statistical analyzes were realized by the Mann-Whitney test. Survival was analyzed by the Kaplan-Meyer survival curve. $P \leq 0.05$ was considered statistically different. **Results and Discussion:** Nasal immunization of mice with PspA5 combined with Bp mutants that either express low levels of different virulence factors (BPLOW) or are defective for the expression of pertussis toxin (PT) (BPRA) induced lower levels of anti-PspA5 IgG than the combination of PspA5 with wP or the wild type BPSM strain. Survival of mice immunized with PspA5-BPLOW or PspA5-BPRA were 60% and 50% respectively, whereas mice immunized with PspA5-wP or PspA5-BPSM showed 83.3% protection. Moreover, immunization of mice with the combination of PspA5 with purified PT stimulated high levels of anti-PspA5 IgG and conferred protection to 100% of mice after the challenge. The results indicate that PT is an important component for the adjuvant activity of Bp in this model.

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4.33 Recognition of linear and conformational epitopes of PspA (Pneumococcal surface protein A) by sera from human volunteers

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Introduction: PspA (Pneumococcal surface protein A) is a promising candidate antigen for the development of protein vaccines against pneumococcal disease. **Objectives:** This work aims to identify linear and conformational epitopes from PspA recognized by the sera of volunteers from an experimental human pneumococcal carriage model developed at the Liverpool School of Tropical Medicine (LSTM). **Methods:** Sera from two groups of volunteers were tested: Cohort A - 10 volunteers that were negative for the presence of pneumococcus at the baseline screening (time 0= T0). These volunteers were then inoculated with a serotype 6B strain and all of them got experimentally colonized. Up to 1 year after this carriage episode, they were re-inoculated with the same strain (time 2 = T2). None of them got re-colonized. Cohort B - 8 volunteers that were naturally colonized with pneumococcus at the baseline screening (T0). They were not inoculated with pneumococcus at this time. Up to 1 year after this carriage episode, they were inoculated with the 6B strain (T2). Half of them got colonized. Two methods were used to identify PspA epitopes recognized by the sera: 1-to identify linear epitopes, peptide arrays containing 15-mer peptides covering the sequences of different PspA variants were used. 2- to identify linear and conformational epitopes, seven fragments (F1-F7) with 100 aminoacids covering the sequence of PspA from strain Rx1 were tested by ELISA. **Results and Discussion:** Using the peptide arrays, sera from the volunteers recognized peptides located at different PspA regions, mainly at the initial N-terminal region and also at the proline-rich region, which is located near the C-terminal end of the protein. Roughly, the same peptides were recognized by the same individual at T0 and T2, indicating that the challenge and colonization events did not alter the linear epitopes recognized by each volunteer. Moreover, we could not determine a peptide that could be used as a marker for protection, since there was no specific peptide recognized only by non-recolonized volunteers after re-challenge. Using ELISA, cohort A showed increased antibody levels to F2, F4 and F6 at T2. Antibodies to these fragments could have a role in protection against re-acquisition of carriage by the 6B strain. For cohort B, antibody levels to both F1 and F7 were increased at T0, which could be a marker for exposure to pneumococci. Our results suggest that conformational epitopes are more important than linear epitopes in the antibody response to PspA after exposure to pneumococci.

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4.34 Evaluation of cross-reactivity between anti-PspA antibodies and the S2 sub fragment of human cardiac myosin

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Introduction: Pneumococcal surface protein A (PspA) is one of the most protective vaccine antigens against pneumococcus in animal models. However, there are concerns on anti-PspA antibodies presenting cross-reactivity with human cardiac myosin. This led to uncertainty about the safety involved in using this protein as a vaccine antigen, since such antibodies could induce cardiac autoimmune disorders, in a similar way to that observed for the M protein of Streptococcus pyogenes. The absence of clinical evidence indicating a relationship between pneumococcal infections and cardiac injuries, argues against this cross-reactivity causing pathological events. **Objectives:** Our study aims to investigate this through two models: one human and another, murine. **Methods:** In the first model, anti-PspA antibodies were purified from the serum of healthy individuals. Subsequently, the reactivity profile of these antibodies to porcine cardiac myosin and peptides from subfragment S2 of human cardiac myosin was assessed by western-blots and ELISA assays. In the second model, sera from mice colonized with *S. pneumoniae* or immunized with the recombinant PspA protein (3 doses of 10 ug of rPspA) were analyzed for the presence of anti-PspA antibodies and their cross-reactivity with the porcine cardiac myosin and peptides from subfragment S2 of cardiac myosin were examined by western blot and ELISA assays. **Results and Discussion:** In the human model, anti-PspA antibodies were quantified by ELISA assays after individual purification of these antibodies from healthy human serum by affinity chromatography. This quantification demonstrated the presence of three groups based on quantity of anti-PspA antibodies in the serum(Q): Q> 50 µg/mL;20 µg/mL>Q> 30 µg/mL and Q=0. Thereafter, western-blot assays showed a low reactivity between purified anti-PspA antibodies and porcine cardiac myosin. Reactivity of these antibodies with the peptides from subfragment S2 of cardiac myosin is being investigated by ELISA. In the murine model, we mimic in mice two conditions: colonization with pneumococcus and immunization with recombinant PspA. In both conditions, production of anti-PspA antibodies was verified by ELISA. After a time course analyses, the peak concentration of anti-PspA antibodies was observed 11 days after the last dose of rPspA in immunized mice; and after 34 days in colonized mice. In western blot assays done with porcine cardiac myosin, serum from colonized mice showed a low reactivity, while the serum of immunized mice presented a significant reactivity with a fragment around 37 kDa, which probably comes from the heavy chain of cardiac myosin.This reactivity is being investigated by ELISA using peptides from subfragment S2 of cardiac myosin.

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4.35 Use of SDS for purification of pneumococcal capsular polysaccharide serotype 14

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Introduction: *Streptococcus pneumoniae* causes infections of human respiratory tract. Among its 93 serotypes described, type 14 is prevalent worldwide and its capsular polysaccharide (PS14) is an important antigen. Traditionally, PS14 has been purified by differential precipitation, chromatography, enzymatic and detergent treatments, and ultrafiltration. Since the purification is responsible for significant part of the production costs of a bioproduct, the development of new purification strategies is essential for minimizing production costs. **Objectives:** Develop a new process for PS14 purification in order to achieve the required purity in terms of proteins (Prt) and nucleic acids (NA) (Prt or NA$\leq$3% w/w relative to PS14 mass) for conjugate vaccine preparation. **Methods:** The cell separation was carried out by tangential microfiltration in 0.22 μm membrane. The microfiltrate was concentrated by tangential ultrafiltration in 50 kDa membrane. The concentrate was washed successively with SDS+EDTA, saline, and Tris bufferin the 50 kDa membrane. The 1st precipitation step was performed with 20% ethanol; after centrifugation, the supernatant was precipitated with 60% ethanol (2nd precipitation). The final precipitate was dissolved in distilled water and centrifuged to obtain the water-soluble PS14. All precipitation steps were performed at 25ºC and centrifugation steps at 10,000 rpm, 45min, 25ºC. PS14 was quantified by sandwich ELISA, total Prt by Lowry and NA by absorbance at 260 nm. **Results and Discussion:** The recovery of PS14 was 70% with 59% Prt and 15% NA after wash steps in 50 kDa membrane. The majority of Prt and NA were eliminated in the 1st precipitation step. Their amount was reduced in 98% and 83%, respectively, but still remained above the requirements (5.8% Prt and 6% NA), with PS14 step recovery of 94%. After the 2nd precipitation and water solubilization, the PS14 step recovery was -0.12% with undetectable amount of Prt and 2.01% NA, therefore achieving the requirements for vaccine production. In previous experiments, the centrifugation steps were performed at 4ºC, leading to great loss of PS14. The centrifugation at 25ºC allowed a global yield of the process of 60%. In conclusion, a new process was developed for PS14 purification, which introduced a wash step with SDS in the ultrafiltration, followed by ethanol precipitations and centrifugation at room temperature. SDS played an important role for elimination of Prt and NA, probably due to denaturation of these molecules, which facilitated their escape through the ultrafiltration membrane and their precipitation by ethanol. Further experiments are necessary in order to verify the reproducibility of the results.

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4.36 Evaluation of immunological parameters in the Radiation-Attenuated (RA) *Schistosoma* vaccine model

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Introduction: Schistosomiasis is an important disease caused by trematode worms of the genus *Schistosoma* affecting more than 200 million people worldwide. One of the major challenges in developing a vaccine against schistosomiasis is the limited comprehension of the mechanisms involved in protection, due to factors related to the complexity of the parasite life cycle and the presence of different mechanisms of immune evasion. **Objectives:** In this sense, this work aims to compare the immune response following a normal infection (infected) and that after immunization with one dose (1V-Th1 response) or three doses (3V-Th2 response) of attenuated cercariae, in an attempt to correlate these with the protection data of worm burden and egg count. **Methods:** We evaluated the immune response of these groups (infected, 1V and 3V) after immunization, and after challenge by peripheral blood cell phenotyping (FACS); measuring the recruitment of cells to the lungs (broncho alveolar lavage); and quantification of cytokines (CBA) and antibodies (ELISA). We also evaluated the protection (worms and egg burden) after perfusion of these animals. **Results and Discussion:** Regarding the peripheral blood cell phenotype, data showed an increase of T $CD4^+$ and $CD8^+$ lymphocytes in the 1V group and B lymphocytes in the infected group at 17 days after immunization/infection. After challenge, there was a reduction of T $CD4^+$ lymphocytes in the 1V group and an increase of B lymphocytes in the 3V group. Concerning the cellular infiltration in the lungs, we observed an increase of lymphocytes, macrophages, neutrophils and eosinophils in the 1V and 3V groups after immunization. The protection data showed a higher reduction of worm burden in the 3V group (61.9%) than in the 1V group (31.5%). Our data showed that the exposure to radiation attenuated cercariae (1 or 3 doses) was capable of eliciting consistently high levels of protective immunity and that this protection is probably related to the cells recruitment to the lung (effector immune response). The next step is to perform microarrays analysis of peripheral blood mononuclear cells (PBMC) from these experimental groups, aiming to observe a correlation between the immune response induced by immunization, the genes differentially expressed and the protection ratio. These results will contribute to understand new mechanisms of action and immunogenicity prediction, allowing the future design of novel delivery systems and adjuvants for recombinant subunit vaccines for schistosomiasis.

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4.37 The protective recombinant BCG-LTA-K63 tuberculosis vaccine induces higher Th1 responses in mice.

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Introduction: *Mycobacterium bovis* BCG has been widely used as a tuberculosis (TB) vaccine, despite waning efficacy in adults. It is considered that BCG is poor in inducing Th1 immune responses for efficient protection against TB. Adjuvant properties have been described for the *Escherichia coli* heat-labile toxin (LT), composed of an enzymatically active A and nontoxic B subunits. **Objectives:** In the present work we investigated recombinant BCG strains expressing LT or LTA-K63 and analyzed the cellular immune response and protection induced by this construct in BALB/c mice. **Methods:** Groups of mice were immunized s.c. with 10^6 colony forming units (CFU) of BCG or rBCG-LTA-K63 and after 60 days the spleens were placed in culture and stimulated with PDS (protein derived from the culture supernatant). After incubation, cytokine secretion was evaluated in the supernatant of splenocyte culture and the cells were collected for intracellular cytokine staining with FITC, PE and PE-Cy7-conjugated monoclonal antibodies against CD4, CD8a, IFN-γ, IL-4, IL-17, TNF-α and IL-2. In another experiment, 60 days after immunization, animals were challenged and after 30 days the lungs were homogenized and serial dilutions were plated for evaluation of bacterial burden (CFU). **Results and Discussion:** Splenocytes from rBCG-LTA-K63 produced significantly higher levels of IFN-γ (1231±120 pg/mL) when compared with BCG (862±259 pg/mL). IL-17 and IL-2 production was comparable in immunized groups and IL-4 was not detected. Mice immunized with rBCG-LTA-K63 contained CD4 T cells producing IFN-γ and TNF-α (47.1 and 34.6%, respectively), in significantly higher proportion than BCG (21.4 and 18.9%) or saline controls (13.0 and 8.4%). Multiple-cytokine-producing T cells were predominantly IFN-γ/IL-2 double producers and levels were significantly higher in rBCG-LTA-K63 cells when compared with BCG. The rBCG-LT and rBCG-LTA-K63 strains induced a 1.5-2.0 log reduction in bacterial load following TB challenge in BALB/c mice. In conclusion, we demonstrated that immunization of mice with rBCG-LTA-K63 induces a Th1-shifted immune response characterized by increased production of IFN-γ and TNF-α, significantly higher than BCG, correlating with a higher protection against TB.

Supported by: Fundação Butantan

4.38 Application of Mass Spectrometry to Identify Contaminant Proteins in Hepatitis B Vaccine

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Introduction: Instituto Butantan's Recombinant Hepatitis B Vaccine contains the purified Hepatitis B Surface Antigen (HBsAg) obtained by culturing genetically engineered *Hansenula polymorpha* yeast cells carrying the surface antigen gene. The harvesting of cells is followed by washing and disruption of the cells to release the HBsAg, which is purified by several physico-chemical steps to eliminate host cell-derived proteins. The final product is submitted to microbiological, biological, and physico-chemical tests, including the purity evaluation by electrophoresis on SDS PAGE. The electrophoretic profile is composed of three major bands, corresponding to the HBsAg monomer (23 kDa), dimer (46 kDa) and trimer (69 kDa), identified by Western Blotting with anti-HBsAg Mabs. Other nonspecific bands could be verified, but they should not exceed 5% of total protein, as required by the WHO. **Objectives:** The aim of this work was toanalyze and identify the nonspecific components verified in one refused batch of vaccine **Methods:** The material was submitted to SDS PAGE, tryptic digestion of protein bands, and electrospray ionization (ESI) mass spectrometric analysis of the resulting peptides, performed using a Bruker model maXis ESI-Q-TOF instrument interfaced with an on-line nanospray source (Bruker Daltonics) to perform LC-MS/MS using a U3000 RSLCnano HPLC (Dionex). The collected data were processed by DataAnalysis/Proteinscape software (Bruker) that automatically submitted the peaklist to MASCOT search program using NCBInr database. **Results and Discussion:** Almost all the proteins identified in the "nonspecific" bands were from the yeasts source, specially, from the dehydrogenase complex, showing that our purification process did not introduce protein contaminants from other source than the host. The mass spectrometry technology proved to be suitable to identify the contaminants since it promoted the knowledge of the physico-chemical characteristics of the contaminant proteins and consequently permitted us to optimize our production process.

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4.39Antimicrobial efficacy of thimerosal in the Hepatitis B vaccine adsorbed formulation

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Introduction: Antimicrobial preservative are substances added to sterile products to protect microbiological growth or from microorganisms that are introduced during repeated use of individual doses. The antimicrobial efficacy of the product either inherently or through the addition of preservatives need to be established for product in multi dose vial presentation. The concentration of preservative can be kept at a minimum if the active ingredients of the formulation possess an intrinsic antimicrobial activity. **Objectives:**This study aimed to demonstrate the efficacy of an antimicrobial preservative, thimerosal, added until 100 mg/mL in the Hepatitis B vaccine adsorbed packaged in multi dose vials. **Methods:** The tests consisted of challenge 3 lots of Hepatitis B vaccine adsorbed, with a prescribed inoculum of *Aspergillus brasiliensis* ATCC ATCC 16404, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 12923 and *Staphylococcus aureus* ATCC 6538 strains. The inoculum was maintained in product at 22.5°C ± 2.5°C, protected from light. The residual antimicrobial activity was removed by use a specific inactivator. The samples were filtered at appropriate intervals 6 and 24 hours, 7, 14 and 28 days and determined the number of viable micro-organisms by membrane filtration. The criteria for evaluation of antimicrobial activity are given in 3 logs reduction in the number of bacteria after 14 days and no increase in yeasts and molds colony forming unit (CFU) of initially inoculated. **Results and Discussion:** The results showed a 3 logs reduction for bacteria in 7 day and no increase in the number of yeasts and molds when compared to the initial inoculum. This study demonstrated the effectiveness antimicrobial protection of thimerosal when added as a preservative in Hepatitis B vaccine adsorbed packaged in multi dose vial.

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4.40Influenza vaccine - validation of split process

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Introduction: Influenza or flu is an acute infectious disease caused by Influenza virus and affects the entire world population. In order to prevent and control its spread the vaccination is the most effective measure. The trivalent seasonal vaccine is updated every year, following to the recommendations of the World Health Organization. The seasonal flu vaccine production at Instituto Butantan is a successful model of technology transfer. In September 2012, after the license of National Regulatory Agency (ANVISA), the Influenza Laboratory started to supply the Ministry of Healthfor the National Immunization Program. The Butantan flu vaccine is hen's embryonated eggs, based and the downstream process requires many steps of purification, split and inactivation. Split vaccine contains basically the surface glycoproteins Haemagglutinin (HA) and Neuraminidase (NA). It causes less local reactions than whole virus vaccines and produces adequate antibody levels. **Objectives:** The aim of this study is to assess and demonstrate that the split step of the bulk production is effective, reliable and reproducible. The validation of this step is one of the requirements of Good Manufacturing Practices (GMP) and has to be evaluated each change of the strain. The process is done adding the non-ionic detergent in the purified virus suspension, disrupting the lipid membrane of the virus into smaller fragments. Influenza virus size ranging from 80 to 120 nm, have sedimentation coefficient of approximately 700 S and locate at 40% of sucrose, during zonal purification. **Methods**: Samples were collected from: a) suspension of purified virus before fragmentation (whole virus) and b) after the addition of detergent (split virus). Both samples were centrifuged into 10 to 60% sucrose gradient at 25,000 rpm for 3 hours. The gradients were fractionated and analysed for protein concentration by Optical Density at 280 nm (OD 280), sucrose concentration (%) and hemaglutination units assay using guinea pig erythrocytes. **Results and Discussion**: In the samples prior to fragmentation (whole virus) the viral peaks were found on high sucrose concentration ($\geq$ 40%) while in the fragmented virus samples the higher concentrations of viral protein are in the lower range of sucrose concentration, when compared both samples. The result showed that viral split step during the production of influenza vaccine is effective and reproducible, attending the requirements of safety, quality and GMP.

Supported by: Fundação Butantan

4.41 Stability Study of Rotavirus Human/bovine used in the Rotavirus Vaccine

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Introduction: Rotavirus is the most common cause of severe diarrhea in children around the world. The development of rotavirus vaccines and the introduction of these into global immunization programs have been high priorities for many international agencies. Rotavirus vaccine is produced with a human attenuated virus, bovine-human or rhesus-human reassortant virus. A new rotavirus vaccine that was developed at Instituto Butantan is composed of five serotypes (G1, G2, G3, G4 and G9) present in Brazil. **Objectives:** To evaluate the stability of rotavirus suspensions used in the pentavalent vaccine when stored at -80°C. **Methods:** Rotavirus suspensions obtained in Vero cell culture infected with Rotavirus human/bovine G1, G2, G3, G4 or G9 with 10% of sucrose buffer were stored at -80°C. Samples of these suspensions were taken on day 0 and after 48 months of storage to determine rotavirus titers by PFA (Plaque Forming Assay) and the results were expressed in PFU/ml. **Results and Discussion**: The results of the samples of rotavirus suspensions showed that after 4 years of storage the titers values found were similar to the initial for the five serotypes. The data found indicate that the sucrose buffer is a good stabilizer for rotavirus human/bovine reassortant.

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4.42 Hydrocortisone effects on Influenza Vaccine production

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Introduction: The flu or influenza is a viral infection that mainly affects the airways. The etiological agent is *Myxovirus influenzae*, family *Orthomyxiviridae*. It is subdivided into types A, B and C according to their surface proteins (hemagglutinin and neuraminidase). The virus exhibits a high mutation rate, often resulting in the insertion of new variants for which the population has no immunity. Transmission occurs easily from person to person when infected people cough or sneeze. In developed countries, annual epidemics of influenza infect about 10-20% of the population each season. Currently the annual flu vaccination is the most effective method to prevent the spread and infection by influenza virus, reducing its complications. However, the global production capacity estimated seasonal flu is approximately 350 million to 420 million doses, and manufacturing laboratories are located mainly in industrialized countries, which suggests a lack of vaccines worldwide. In case of a pandemic, because of the lack of pre-existing immunity, number of doses or larger amounts of antigen will likely be required against the new strain. In this scenario there are demonstrations, little explored, that the course of infection of influenza virus in chicken embryos is affected by the administration of corticosteroid hormones. **Objectives:** The effect of hydrocortisone as enhancer of viral replication was evaluated in order to determine the optimal concentration and to establish systematic procedure for the analysis of different viral strains of influenza virus. **Methods:** Some steps of the production of influenza vaccine were simulated on small scale with different concentrations of hydrocortisone on viral inoculum. During the experiments the data as mortality of the embryos and volume of allantoic fluid harvested were evaluated. The concentration of viral hemagglutination was verified by single radial diffusion and SDS-page technique. **Results and Discussion:** The use of hydrocortisone as potentiator of viral replication has shown promise for the strains of type B and low expressivity or the reverse effect was observed with strains of type A. The optimal concentration varied according to the strain. For B/Wisconsin/1/2010 strain was 10 µg/mL for the B/Brisbane/60/2008 was 40 µg/mL and A/California/7/2009 (H1N1) was 60 µg/mL. Under the experimental conditions we were able to determine the optimal hydrocortisone concentration that can be used to enhance the vaccine production.

Supported by: Fundação Butantan

4.43 Effect of the filtration in the dengue virus suspension

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Introduction: Dengue is a common viral disease of humans that has become a major international public health concern. About 3.5 billion people are living in dengue-endemic countries. This disease is an important public health problem in Brazil mailing the hemorrhagic dengue. Nowadays the major challenge to researchers is the development of a good vaccine to prevent this disease. **Objectives:** The aim of this study is to determine the influence of the membrane porosity in the titer of virus dengue suspension used in the Butantan dengue vaccine production. **Methods**: Eight virus dengue infections (2/serotype) in Vero cells were realized. For it were used the NIH strains DEN1Δ30, DEN2/4Δ30, DEN3Δ30/31 and DEN4Δ30. After eight (DEN1, DEN2 and DEN4) and eleven days (DEN3) of incubation at 36.5°C the supernatant of these cultures were harvest and filtered in two different PVDF membranes porosity (0.22 and 0.45 µm). Samples were taken to determine the viral titers by PFA (Plaque Forming Assay) and the results were expressed in PFU/ml.**Resultsand Discussion:** The virus titers found in the 24 harvest (8/serotype) were similar. The results showed there wasn't virus absorbency in both membranes (0.22 and 0.45 µm). These data indicate that the PVDF membranes are good for dengue virus filtration.

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4.44 Comparative study between two immunization schemes using rabies vaccine associated with BpMPLA-SE adjuvant

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Introduction:The rabies vaccines produced at Butantan when associated with BpMPLA-SE adjuvant increase the immune humoral response in mice. **Objectives:** Evaluate the immune response for this vaccine using two immunization schemes, one with injections on days 0, 7 and 28 (S1) and other on days 0 and 21 (S2). **Methods:** Four groups (G1, G2, G3 and G4) with eight Balb/c mice were used in this experiment. Two groups received 500 µL of rabies vaccine with 10 µg BpMPLA-SE adjuvant, using the schemes S1 (G1S1) and S2 (G2S2). The other groups were immunized with only 500 µL of rabies vaccine using S1 and S2 schemes (G3S1 and G4S2). Blood samples were taken on days 60, 120 and 180 after finishing the vaccination to determine the titers of neutralizing antibodies for rabies virus in BHK21 cells (RFFIT). **Results and Discussion:** The averages of the neutralizing antibodies titers found in the samples from each group 60 days after finishing the immunization were 18.5, 26.9, 13.0 and 14.0 IU/ml for groups G1S1, G2S2, G3S1 and G4S2 respectively. The results obtained on day 120 were 17.8 (G1S1), 22.5 (G2S2), 10.9 (G3S1) and 13.1 IU/ml (G4S2) and for day 180 the titers founded were 17.1 (G1S1), 15.7 (G2S2), 9.9 (G3S1), and 10.4 IU/ml (G4S2). The antibodies titers in mice immunized with rabies vaccine and adjuvant BpMPLA-SE using two schemes was higher than the titers obtained in mice immunized with vaccine only. The results indicate that the S2 (2 doses/vaccine) induced an immune response for rabies similar to S1 and more efficient when the vaccine was associated with adjuvant. In conclusion, the scheme 2 and the use of adjuvant BpMPLA-SE are important strategies to reduce the costs of rabies immunization.

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4.45Probiotic increases the antirabies immune response in cattle after vaccination

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Introduction: The rabies vaccination for bovines is recommended in Brazil to control of rabies disease in these animals. However, in this country there are regions where the immunization of the animal with two doses of rabies is impossible because of the costs of this procedure. **Objectives:**In this study was evaluated the effect of a probiotic in the immune response of cattle immunized with one dose of rabies vaccine after receiving mineral salt with probiotic. **Methods:** Thirty cattle were divided randomly into 2 groups, one (G1) was immunized with one dose of commercial rabies vaccine after receiving70 g of mineral salt with 4 g of DBR SACCH probiotic (IMEVE-Biotec-SP, Brazil)/animal/day during 90 days. Other group (G2) received the same dose of vaccine and 70 g of mineral salt/animal/day. Samples of blood of the animals were taken on days 0, 42 and 73 after vaccination to determine the neutralizing anti-rabies antibodies in BHK21 cells (RFFIT). **Results and Discussion:** The geometric means of the titers obtained in the samples of the G1 were 0.06, 1.80 and 1.28 UI/mL on days 0, 42 and 73 respectively. The results found in the animals of the G2 were 0.06, 0.55 and 0.20 UI/mL on days 0, 42 e 73. These data show that the probiotic increases the anti-rabies titer 3.2 times 30 days after vaccination and 6.4 times after 60 days. The results indicate that the use of the 4 g/animal/day of probiotic DBR SACCH increases the immune response for rabies.

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4.46 Cellular immune response of mice vaccinated with recombinant rabies virus glycoprotein (rRVGP) and mRNA coding for rRVGP

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Introduction: Rabies is still responsible for 55 thousand deaths around the world. Most cases happen frequently in developing countries that don't have access to new vaccines and consequently are still using less effective vaccines. Facing this reality, the development of a cheaper and effective vaccine, as well as post exposure treatment is still important. Protection against rabies virus infection is attained by the induction of both humoral and cellular immune response. **Objectives:** Here in this study we have evaluated the cellular immune response induced by recombinant rabies virus glycoprotein (rRVGP) and vector-delivered mRNA (SFV-RVGP) antigen preparations by evaluating the pattern of cytokines induced upon immunization. **Methods:** we have immunized BALB-c mice, i.p., 3 doses (days 0, 7 and 14) with: commercial rabies vaccine, SFV-RVGP, S2 insect cells derived rRVGP, inactivated rabies virus and SFV-RVGP (priming, 1x) associated with rRVGP (booster, 2x). At day 21 the spleens of the animals were removed, cultivated at culture microplate for 72 h and stimulated or not with inactivated rabies virus. The supernatants were then collected and analyzed by ELISA. **Results and Discussion:** results showed that only animals that were previously immunized with commercial rabies vaccine and SFV-RVGP synthesized good amounts of IL-2 (>300 pg/mL). The IL-10 expression achieved values above 1000 pg/mL just in the group immunized with rabies vaccine. The IL-12 showed values above 1,000 pg/mL only in the group that received only SFV-RVGP vaccine. The IFN-γ production showed values above 700 pg/mL in mice immunized with vaccine and SFV-RVGP. We detected a good amount of IL-4 (~160 pg/mL) only in vaccine group. The presence of Alumen adjuvant in the commercial rabies vaccine confers to this antigen the property of lymphocytes proliferation/activation. The absence of adjuvant in the rRVGP preparations made them a weaker immune response inducer. The RVGP-mRNA immunization by the delivery of infective SFV-RVGP efficiently activated cellular immune response, mainly, as shown in previous studies, by the infection process leading to the formation of apoptotic bodies and cross-priming antigen presentation. The results showed that SFV-RVGP can be a good vaccine candidate against rabies and for post exposure treatment (alone or associated with rRVGP) as it induces high levels of IL-2 synthesis. Despite previous demonstration of the activity of S2 cells derived rRVGP on stimulating the synthesis of neutralizing antibodies, cytokine analysis showed poor activation of lymphocytes. This immunological response pattern would benefit of a formulation containing adjuvant for immune response enhancement.

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5. Microorganisms

5.01 Role of the serine protease Pic in atypical EPEC intestinal colonization

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Introduction: The Protein Involved in Colonization (Pic) is an autotransporter protein described in enteroaggregative *E. coli* (EAEC). In EAEC Pic is responsible for proteolytic activity on mucin, serum resistance and hemagglutination. The Pic-encoding gene (*pic*) was detected in one strain of our enteropathogenic *Escherichia coli* (aEPEC) laboratory collection.**Objectives:** Characterization of Pic expressed by one aEPEC strain.**Methods:** aEPEC strain BA589 (serotype O5:H2) isolated from a case of infantile diarrhea was studied. Pic expression was detected by immunoblotting of culture supernatants. Plasmid extraction analysis was followed by Southern blot with a probe corresponding to the *pic* sequence of EAEC strain 042. Nine pairs of primers with overlapping regions were designed in order to cover the entire *pic* sequence. The corresponding amplicons were sequenced and the contigs were assembled as a unique sequence for further analysis. The ability of aEPEC BA589 to colonize the intestinal mucosa was evaluated using the streptomycin-treated mice model.**Results and Discussion:** Pic expression was detected in aEPEC BA589 using antiserum raised against Pic of EAEC 042. The Pic-encoding gene (*pic*) in aEPEC BA589 was located in a high-molecular-weight plasmid (~98 Kb). DNA sequence analysis of *pic* from aEPEC BA589 (4,119 nucleotides) showed identity of 99% with *pic* of EAEC 042. Also, BA589 was able to colonize the intestinal mucosa of streptomycin-treated mouse for 15 days. This is the first description of Pic expression in a diarrheagenic *E. coli* pathovar other than EAEC. Pic in BA589 is plasmid-encoded and might be involved in intestinal colonization.

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5.02 A multiplex PCR assay, an effective tool to diagnosis of typical and atypical enteroaggregative *Escherichia coli*

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Introduction: The diarrheagenic *Escherichia coli* pathotype known as enteroaggregative *E. coli* (EAEC) is defined by the expression of the aggregative adherence pattern (AA) on cultured epithelial cells. The presence or absence of the *aggR* gene, located in the EAEC virulence plasmid, defines EAEC as typical or atypical, respectively. The gold standard diagnostic for EAEC is the observation of the AA pattern in adherence tests with cultured epithelial cells. However, this assay is expensive, time consuming and needs specific laboratory infrastructure. Molecular diagnostic assays have been developed as alternatives to the adherence tests. These tests detect plasmid markers not suitable for atypical EAEC diagnosis. **Objectives:** The present study aimed the standardization of a multiplex PCR for the molecular diagnosis of typical and atypical EAEC.**Methods:** The following EAEC genes were selected for the multiplex composition: *aggR*, *aatA* (plasmid-encoded), *aaiA* and *aaiG* (chromosomally encoded). Initially, the multiplex reaction was set up using purified DNA of EAEC prototype strain 042 as positive control and *E. coli* K12 as negative control, and the detection limit was determined. The multiplex reaction was employed to evaluate a collection of 103 EAEC strains previously characterized. Later a blind test using multiplex PCR was performed with 403 strains of fecal *E. coli* previously classified as belonging to diarrheagenic *E. coli* pathotypes or as non-pathogenic, in order to determine the sensitivity and specificity of the assay. **Results and Discussion:** The test was successfully standardized using prototype EAEC strains and its detection limit was 125 ng of purified DNA. Then, the reaction was evaluated in a collection of 403 strains of fecal *E. coli* previously classified as belonging to diarrheagenic *E. coli* pathotypes or as non-pathogenic. The technique presented 94.8% of sensitivity, 94.5% of specificity, 74.3% of positive predictive value and 99.1% of negative predictive value. Moreover, there was an increase of 60% in detecting atypical EAEC. Therefore, the test developed is a sensitive and specific tool for the diagnostic of typical and atypical EAEC employing isolated colonies of *E. coli* from stool cultures.

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5.03 Isolation and whole genome analyses of Brazilian pathogenic *Leptospira interrogans* serovar hardjo subtype hardjoprajitno

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Introduction*:* *Leptospira interrogans* serovar Hardjo subtype hardjoprajitno is the most prevalent pathogenic serovar in bovine leptospirosis in Brazil. The disease is associated with abortion, stillbirths and reduction of milk production worldwide. In Brazil, three cattle clinical isolates of the subtype hardjoprajitno were obtained from two leptospirosis outbreaks in dairy farms. The strain Norma represents one of those isolates included in Brazilian vaccine formulation for leptospirosis control. **Objectives**: To improve our knowledge on the taxonomic classification and genomic features of the subtype hardjoprajitno classified in *L. interrogans* species. **Methods**: The genome was accomplished using the 454 sequencing with paired end tags and shotgun methodologies. DNA assembly was performed by combining Newbler software package, FGAP and Jcontigsort. **Results and Discussion:** In chromosome I, we found 4.406.716 bases comprising approximately 1.250 hypothetical proteins, 18 truncated and 2.750 coding sequences with putative assigned function. Additionally, chromosome II contains a total of 376.516 bases comprising 326 genes with predicted function. Through comparative analyses, we identified 2 complete and 2 partial copies of virulent associated *LigA* and *LigB* genes and two integral copies of *MCE*-like protein. Furthermore, mobile elements were identified suggesting association with recombination events in genomic features. By analyzing the O-antigen gene cluster (rfb locus), we identified nucleotide sequence homolog of ORF19 from hardjobovis in hardjoprajitno rfb locus that is absent in other *L. interrogans*, suggesting evolutiveacquisition of this sequence from this serovar. As expected, we confirmed the presence of ORF36 in the genomic region of serovar Hardjo subtype hardjoprjitno. *Leptospira interrogans* serovar Hardjo subtype hardjoprajitno harbor differences in *rfb* locus and virulent coding genes, being some of which, potentially linked to specific features of leptospirosis disease and leptospires evolution.

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5.04 Cloning and expression of Hfq protein

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Introduction: *Host factor for the replication of phage Qβ* (Hfq) is an 11kDa protein that has a role in gene regulation. Primarily described as a factor for phage Qβ transcription, it regulates several cellular processes, including stress response, decreased virulence and quorum sensing. Hfq post-transcriptional regulation is due to its capacity of pairing *small RNA* (sRNA) to near-complementary sequences in target *messenger RNA* (mRNA). Recent studies have been shown that Hfq acts on the following phenotypes: motility in *Escherichia coli* (Enterohaemorrhagic *E. coli*, Enteropathogenic *E. coli*, Uropathogenic *E. coli*), *Salmonella enterica* serovar Typhimurium, *Klebsiella* sp; pathogenesis in EHEC, EPEC, *Salmonella enterica* serovar Typhimurium, *Klebsiella* sp, *Vibrio* sp; biofilm formation in *Salmonella* sp; besides its regulation on genes related to cellular stress. **Objectives:** Cloning and expression of Hfq protein from Enteropathogenic *Escherichia coli*. **Methods:** Hfq gene was cloned into pGEM T-Easy and subcloned into pET 28a vector. **Results and Discussion:** Results were confirmed by PCR using primers based on the Hfq sequence as previously described, and sequencing. Recombinant protein purification by chromatography affinity, antiserum production and immunologic assays are set as future goals for this study.

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5.05 Is *Quorum Sensing* involved in biofilm formation by atypical enteropathogenic *Escherichia coli*?

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Introduction: Atypical EPECs form biofilm on abiotic and pre-fixed cell surfaces. *Quorum Sensing* (QS) systems controls diverse mechanisms in *E. coli*, including expression of virulence factors and biofilm formation. QS is a signaling system which confers to some bacteria the ability to respond to chemical molecules, known as autoinducers (AI). Bacteria are capable to produce, release, detect and respond to these molecules. When these compounds reach critical concentrations, transcriptional factors are activated, altering gene transcription. SdiA receptor is responsible for AI-1 detection, but *E. coli* do not produce AI-1 detecting therefore AI-1 produced by other bacteria. The QseC receptor is responsible for the detection of an aromatic compound, known as AI-3, produced by the bacteria. QseC is a histidine kinase sensor that also has the ability to respond to human hormones epinephrine and norepinephrine, sugesting its involvment in inter kingdom signaling. **Objective**: The aim of this study was to verify the role of *sdiA* and *qseC*, in aEPEC biofilm formation. **Methods**: *sdiA* and *qseC* mutants were obtained through homologous recombination and biofilm formation were analyzed in LB medium through 8 and 24 hours of incubation, under 30° C and 37° C, using the crystal violet colorimetric assay in polystyrene microtiter plates. **Results and Discussion:** ONT:H25 *sdiA* mutant had statistically greater biofilm formation comparing to wild type strain in both conditions. However, O55:H7 *sdiA* mutant produced higher biofilm formation comparing to O55:H7 wild type strain only at 37° C, and 24 hours of incubation. Analysis of *qseC* strains noticed a decrease in biofilm formation by O55:H7 *qseC* mutant in 24 hours comparing to O55:H7 wild type, while ONT: H25 *qseC* mutant had decreased biofilm formation comparing to wild type in the period of 8 hours, both at 37 ° C. In other conditions, mutant strains were very similar to wild type ones. The data obtained until now reveal that *sdiA* and *qseC* genes are involved on biofilm formation, suggesting that QS can be involved in biofilm regulation by aEPEC.

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5.06 Regulation of Atypical *Escherichia coli* Attaching and Effacing lesion by bacterial adrenergic sensor kinase QseE

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Introduction: Enteric pathogens use the mammalian hormone epinephrine as a signal for differential regulation of the virulence factors, including motility, invasion and attaching and effacing (A/E) lesion formation. The A/E lesions are typical of EHEC and enteropathogenic *E. coli* (EPEC) infections and are characterized by the attachment of bacteria to colonic epithelial cells followed by an induction of extensive actin rearrangement underneath the bacteria and effacement of surrounding microvilli. Two histidine- kinase sensors have been identified as sensors of epinephrine in EHEC. The first, QseC, has been reported to increase its autophosphorylation in response to epinephrine, norepinephrine, or bacterial autoinducer 3 (AI-3). The second sensor, QseE, responds to epinephrine, phosphate, and sulfate. QseE has been reported as a negative regulator of the expression of the Locus of Enterocyte Effacement (LEE) of EHEC, the pathogenicity island responsible for A/E lesion formation. Although the role of epinephrine QseC-dependent regulation of A/E lesion formation of EPEC has been previously investigated, the effect of this hormone on QseE-dependent regulation has not been determined in this patotype. **Objectives:** To investigate the role of QseE sensor in the regulation of A/E lesion formation in atypical EPEC. **Methods:** atypical EPEC (aEPEC) strain lacking *qseE* gene was generated using the *lambda-red* recombination method and analyzed in the presence and/or absence of epinephrine by fluorescent actin staining (FAS) to test its ability of forming A/E lesion in HeLa cells. **Results and Discussion:** The aEPEC *qseE* mutant strain was successfully obtained. FAS test has shown a slightly increase in the ability of forming A/E lesion in the *qseE* mutant strain compared to wild type strain. The coincubation of HeLa cells with epinephrine has shown an increase in aEPEC infectivity. In this work, we show that the adrenergic kinase QseE regulates the *in vitro* A/E lesion formation in aEPEC, probably acting as a negative regulator of the expression of LEE genes in this enteric pathogen. Further experiments will be performed to confirm this role at transcriptional levels.

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5.07 Detection of toll-like receptor 2 transcrips in lungs of mice infected by pathogenic *Leptospira* sp

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Introduction: Leptospirosis is a zoonotic disease widely disseminated worldwide. The disease is caused by spirochetes of the genus *Leptospira* that infect humans and animals. Among the pathogenic species, *L. interrogans* is the most important cause of the disease in humans. In Brazil leptospirosis is considered endemic with seasonal epidemic outbreaks, representing a serious public health problem. The bacterium spreads via the circulatory system, colonizing the target organs. The pathogenesis in humans is observed mainly in the lung, with cases of pulmonary hemorrhage and acute respiratory failure, lesions in liver tissue and kidneys, leading to renal failure. The initial interactions between the pathogen and host cells induce an innate immune response at the infection sites. Furthermore, Toll-like receptors (TLRs) have been shown to play a central role in the recognition of bacterial components, and it was associated to the intracellular signal pathways in the expression of numerous inflammatory cytokines and chemokines. Several in vivo and in vitro studies have assessed the damage caused by infections with *L. interrogans*, but the rules of the toll-like receptors on the innate immune response and its mechanisms of action on leptospirosis is not well known. **Objectives:** In the present study the toll-like receptor 2 (toll-2) transcripts profile in lungs, of infected mice was investigated. **Methods:** The virulence of *L. interrogans* was maintained by inoculation in golden hamsters (*Mesocricetus auratus*). The bacteria was isolated from the organs (liver, kidney and lung) of the animals and cultured in EMJH medium. Mouse strain C3H/HeJ and BALB/c mice were infected intraperitoneally with $1x10^7$ bacteria, the virulent *Leptospira interrogans* serovar Copenhageni. Three mice for each strain (n=12) were sacrificed on days 1, 3, 5 and 7 after infection. Three uninfected animals of each strain were sacrificed at day 0 as control. Total RNA was isolated from the lungs, quantified and used to prepare cDNA. The transcripts of toll-2 receptor and β-actin were detected by semiquantitative reverse transcription (RT-PCR). **Results and Discussion:** Our preliminary results indicated that higher amounts of toll-2 transcripts were induced in lungs of BALB/c mice when compared with C3H/HeJ mice after the infection with *L. interrogans.* This preliminary data provides information on the profile of toll-2 in leptospirosis infection. Considering the known higher resistance of BALB/c mice to leptospirosis, this data suggests that toll-2 is important in the picture of protective immune response. Better knowledge on toll-like receptors profile after *Leptospira* infection of resistant and sensitive mice strains may contribute for the elucidation of the mechanisms of pathogenesis in leptospirosis.

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5.08 Cell display system for the expression of leptospiral lipoproteins on the surface of *E.coli*

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Introduction: The display of proteins or peptides on the surface of microbial cells has a wide range of biotechnological applications, including vaccine development, bioadsorbents for removal of harmful chemicals and heavy metals, biocatalysts and biosensors by anchoring enzymes, receptors or other signal-sensitive components, antigen delivery and screening of peptide or protein libraries. Several cell-surface display systems have been described in the literature. Most of them are based on anchoring motifs present on the outer membrane proteins, lipoproteins, secreted proteins or subunits of surface appendages of bacteria. **Objectives:** In the present study, we aimed to construct a cell display system for the expression of leptospiral lipoproteins on the surface of *E. coli* using the signal sequence (Lpp) and lipoprotein signal peptidase recognition site (LSP) of the major *E. coli* lipoprotein. **Methods:** Four oligonucleotides were synthesized, that when annealed and ligated encode the *E.coli* Lpp signal sequence, LSP recognition site and outer membrane targeting signal. The construct was designed with overhangs to allow direction cloning into NdeI and XhoI digested pET-37b(+) vector. Five restriction sites were included downstream of the outer membrane sorting signal to provide a multiple cloning site. This new vector was named pLIPO. To test the capacity of this system to express lipoproteins, four genes encoding probable outer membrane lipoproteins from *Leptospira interrogans* sorovar Copenhageni were cloned into the pLIPO. **Results and Discussion:** The recombinant lipoproteins were expressed and characterized by globomycin assay and their surface expression was detected by FACS analysis. Our results demonstrated that mature portions of four leptospiral lipoproteins cloned into the pLIPO vector were expressed, lipidated and exposed on the surface of *E.coli*. This new cell display system for the expression of leptospiral lipoproteins on the surface of *E.coli* can potentially be applied for identification of novel antigens for the development to vaccine and diagnostic methods.

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5.09 Bis-(3´-5´)- cyclic guanosine monophosphate (c-di-GMP) participates in the regulation of motility and biofilm formation in atypical enteropathogenic *Escherichia coli* (aEPEC)

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is one of the etiologic agents of persistent diarrhea in children and have become the most common bacterial pathogens in our environment that causes diarrhea. The adhesion on epithelial cells forming microcolonies is a hallmark of EPEC, and this feature is thought to enable biofilm formation. Biofilms are associated with bacterial persistence and resistance to antibiotics. Atypical EPEC (aEPEC) of different adherence patterns are capable to form biofilms on abiotic and cell surfaces. Recently, several studies have pointed Bis-(3' -5')- cyclic guanosine monophosphate (c-di-GMP) as an important mechanism involved in signaling transition from planktonic state to bacterial biofilm. YcgR binding protein is directly involved in the regulation of flagellar movement through the binding of YcgR/c-di-GMP complex to flagellar proteins, which reduces flagellar movement. **Objectives:** The aim of this study was to evaluate the influence of *ycgR* gene in motility and biofilm formation in aEPEC. **Methods:**Biofilm formation in the O55:H7Δ*ycgR* mutant was analyzed by colorimetric assay at different time periods (2h, 4h and 6h) growing on LB medium. The motility test was carried out in 0.3% agar plate test and the growth was measured after 6 hours incubation at 37 °C. **Results and Discussion:** The results demonstrated a decreased biofilm formation in the O55:H7Δ*ycgR* mutant compared to the wildtype strain. The motility was also reduced in the mutant strain. These results suggest that c-di-GMP may be involved in the regulation of biofilm formation in aEPEC, and YcgR is part of this signaling cascade, corroborating previous studies.

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5.10 Detection and serological analysis for influenza virus in anseriformes from Municipal Parks of São Paulo

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Introduction: Waterfowl migration, particularly birds of the order Anseriformes, are considered the main reservoir of influenza virus. All 16 hemagglutinin and 9 neuraminidase were isolated from these birds, which shed the virus in feces and oropharyngeal secretions. From these aerosols, the virus can be transmitted to other hosts, including man, especially in environments that favor the interaction between species. In São Paulo, these environments are represented by the Municipal Parks that apart from establishing leisure environments for people, home to a huge diversity of animal species. **Objectives**: Identify the presence of influenza virus circulating in the population of domestic Anseriformes present in the Municipal Parks of São Paulo. **Methods**: We evaluated 48 samples of oropharyngeal and 50 cloacal samples, totalling 98 swabs from 65 birds, among them 5 Black Swans (*Cygnus atratus*), 21 geese (*Anser* sp.), 21 Teals Mallard (*Anas platyrhynchos*) and 9 Teals Beijing (*Anas platyrhynchos*). The swabs were evaluated by molecular analysis using the technique of RT-PCR using the kit Promega GoTaq with a pair of primers for the matrix protein of the influenza virus M1 and M2, then routing for ultrastructural analyzes. The 47 samples collected were analyzed by the technique of the hemagglutination inhibition (HI) for subtypes A/H1N1, A/H3N2, A/H7N7 and A/H3N8. **Results and Discussion**: The serology showed that over 90% of animals exhibit antibodies against these subtypes. Electrophoresis bands were observed in 8 specimens, with a length of approximately 300 bp obtained from 6 animals with 5 samples oropharyngeal and 3 samples cloacal swabs. Positive samples (cDNA) were sequenced and evaluated by the method Blast, whose results confirm that this is the type of influenza virus A with 97% similarity with subtypes of type A virus (H7N7) and (H3N8). The results suggest the presence of influenza virus in waterfowl of the parks. The number of animals with potential virus circulation it is higher, considering that viral clearance occurs all day and also they are in constant contact with animals free-living, wild animals and humans, then it is important to implement a monitoring system to prevent the emergence of epidemic outbreaks.

5.11 Functional characterization of a probable collagenase from *Leptospira interrogans*

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Introduction: Leptospirosis is a zoonosis widespread throughout the world, caused by pathogenic spirochetes of the genus *Leptospira*. Transmission occurs through direct contact with water and soil contaminated with urine of infected animals. Pathogenic leptospires invade host tissues by penetrating damaged skin or the mucous membranes. After entering the host, the progression of the infection involves the adhesion of bacteria to eukaryotic cells and extracellular matrix proteins followed by invasion of tissues. The mechanism involved in pathogen invasion through extracellular barriers is not well elucidated. Enzymes capable of degrading extracellular matrix proteins could contribute to motility and chemotaxis of bacteria during these events. Pathogenic bacteria synthesize and secrete different types of proteases that degrade host proteins. The experimental proof of the existence and functional characterization of these proteins may contribute to the understanding of the pathogenesis of leptospirosis. **Objectives**: This work aimed the cloning, expression and functional characterization of a probable collagenase from *L.interrogans* serovar Copenhageni. **Methods**: Coding sequences of the collagenase domain 1 (D1), collagenase domain 2 (D2), and both domains (Full) of the ColA gene were cloned into the pAE expression vector. The recombinant 6xHis-tagged protein fragments were purified by nickel affinity chromatography. The purified fragments were used to obtain the polyclonal antiserum, and their enzymatic activities were evaluated by zymography. **Results and Discussion**: D1, D2 and Full fragments of ColA protein were expressed in *E. coli* and purified from the insoluble fractions. Rabbit polyclonal antiserum against the recombinant protein fragments were produced with a high antibody level detected by ELISA. Western-blotting experiments demonstrated the presence of ColA protein in different pathogenic serovars of *Leptospira*. Zymography revealed that ColA protein is able to degraded gelatin. These results indicated that ColA is probably a leptospiral protein involved in invasion of host tissues.

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5.12 Anti-phagocytosis Induced by Atypical Enteropathogenic *Escherichia coli* (aEPEC): Secretion of the Active Component in Defined Media

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Introduction: Our group has shown that aEPEC (O55:H7) grown in TSB medium secretes a factor which is able to inhibit phagocytosis of *E. coli,S. flexneri*, *S. cerevisiae* and inert latex particles. The isolation and identification of the component(s) responsible for the effect depends on previous fractionation, such as by solid phase extraction (SPE) and High performance liquid chromatograph (HPLC). However, the complexity of TSB medium prevents both isolation and identification of the active component. **Objective:** To investigate if the secretion of the aEPEC anti-phagocytic factor occurs in defined composition media. **Methods:** aEPEC was grown in the following defined media: minimum medium (M9) and Dulbecco's modified eagle medium (DMEM). The supernatants from the bacterial cultures were fractionated by C18-SPE. The active fraction was then either re-fractionated by HPLC or treated with trypsin and Chymotrypsin. J774.A1 macrophages were incubated with the different HPLC fractions and the enzymatically treated material and tested for the anti-phagocytic activity using a control bacterial sample. **Results and Discussion:** The anti-phagocytic activity was detected in cells incubated with an HPLC fraction of the DMEM grown bacteria supernatant eluted with a retention time between 19 and 22 min.On the other hand, in the supernatant from bacteria grown in M9 the anti-phagocytic activity could only be detected when using twice the concentration used for the fractionation of DMEM grown bacteria supernatant. M9 is a poor medium, lacking much of the nutrients present in DMEM, such as amino-acids and vitamins, which may explain the low secretion of the anti-phagocytic activity when compared to the supernatant from bacteria grown in DMEM. In addition, treatment of the active material with trypsin and chymotrypsin helped determine the nature of the anti-phagocytic factor(s). Only the fraction treated with trypsin lost its activity. Trypsin specifically hydrolyses peptidic bonds at the carboxyl side of residues with positively charged side chains, such as arginine and lysine. From these results we conclude that aEPEC is capable of secreting the anti-phagocytic factor, which is very likely of peptidic nature. We also show that a reasonable supply of nutrients is important for its efficient secretion, since the supernatant from DMEM grown bacteria is more active than the supernatant from M9. The use of defined media, richer than M9, but less complex than TSB, should facilitate the identification of the active molecule in fractions analyzed by HPLC.

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5.13 Detection of O157 *Escherichia coli* strains by immunodot assay

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Introduction: Diarrhea is a multiple causes disease, but diarrheagenic *Escherichia coli* is considered the main bacterial agent. This pathogen is subdivided into six categories according to their serotype, virulence mechanisms and clinical manifestations. Among the 179 serogroups, the O157 serogroup is composed mostly by Shiga toxin-producing *E. coli* (STEC), which constitutes a serious public health problem being related to severe symptoms such as hemolytic uremic syndrome (HUS) in children under 5 years, and hemorrhagic colitis and thrombocytopenia in adults. In relation to diagnosis *E. coli* is somewhat evidenced by clinical practice as well as through the severe symptoms caused mainly by serogroup O157, thus a rapid and accurate diagnosis is necessary. **Objectives:** Immunodot standardization for detection of strains of *E. coli* O157 serogroup using an anti-O157 IgM-monoclonal antibody. **Methods:** To standardize the immunodot EDL933 (O157:H7) bacterial growth was submitted to lysis in which we added 0.2 mg/mL polymyxin B and 2% Triton for 5 min at room temperature and pellet ressuspended in 0.01 M PBS pH 7.3 and heated at 100 °C for 5 and 10 min. Before these treatments bacterial growth was adjusted to 1 OD and 100 µL, 200 µL, 300 µL, 400 µL and 500 µL of antigen were applied onto nitrocellulose membrane. In order to select the smallest concentration of anti-O157 IgM-monoclonal antibody 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL and 60 µg/mL were applied for antigen detection. **Results and Discussion:** Our results showed that either 0.2 mg/mL polymyxin B or heated suspension had a similar ability to promote the release of the target antigen, thus we employed the heated suspension as it provided better cost-benefit ratio. The best fitness of immunodot was when we applied 400 µL of the antigen and 40 µg/mL of the antibody. Thus, these results suggested that the immunodot assay can be a promising alternative for diagnosis, as all tested O157 isolates were detected and non-O157 were not recognized.

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5.14 Biofilm formation by atypical EPEC strains on Calgary biofilm device and on microtiter plate in different growth conditions

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Introduction: Atypical EPEC (aEPEC) has been characterized as the main bacterial agent who causes acute diarrhea in children living in developing countries. Biofilms are structures formed by sessile microorganisms embedded in an extrapolimeric matrix and attached to a surface, associated with bacterial persistence. Flat-bottom microtiter plates (MTP) have been largely used in biofilm formation assays due to its easy manipulation and low cost. The Calgary biofilm device (MBEC – "Minimum biofilm eradication concentration") has been applied in antimicrobial assays and to verify the biofilm formation. **Objectives:** The aim of this study was to compare these two different types of bases in the study of biofilm formation by six strains of aEPEC in different growth conditions through a colorimetric assay. **Methods:** The *E. coli* strains were grown at 26° C and 37° C in quasi static conditions in Luria Bertani medium (LB), Luria Bertani without NaCl (LB without NaCl), M9 minimum and *E. coli* broth. After 72 hours of incubation, the biofilm mass was quantified using crystal violet staining assay and this absorbance was measured at 595 nm.**Results and Discussion:** The strains were capable to form biofilm in polystyrene MTP and MBEC bases, highlighting strains BA 4157, LB 3.25 and LB 5.10, mainly at LB without NaCl and *E. coli* broth. The *E. coli* strains were capable to form biofilm at 37° C and at 26° C, pointing the capacity of maintenance in the environment. The material analyzed in MTP may be composed by planctonic bacteria which aggregate and deposit on the bottom of that device joining the ones in the biofilm in a manner they cannot be separated and distinguished. This fact can explain the greater biofilm formation in MTP by three strains growth in *E. coli* broth. In MBEC, the strains form biofilm on the surface of the pegs where they are immersed in the medium and this material is the real biofilm to be studied. The biofilm formation (OD at 595 nm) in MTP varied from 0.120 to 3.408 whereas in MBEC the values were from 0.040 to 3.397, with certain similarity between the bases. Through the scanning electron microscopy of some strains in MBEC, we verified that a large number of bacteria adhered on the superior part of the peg and less adherence on the inferior part. The aEPEC strains presented a heterogeneous biofilm and it can present its real capacity of biofilm formation when MBEC is used. Adhesins or any other structures can be influencing in different ways between the strains and in each culture condition, resulting in different phenotypes.

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5.15 Antimicrobial activities against atypical EPEC pre-formed biofilms

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Introduction: The capacity of adhesion and biofilm formation by atypical EPEC (aEPEC), one of the main bacterial agents of acute diarrhea in children from developing countries, possibly plays a role in pathogenesis, mainly in bacterial persistence or recurrence of an infection. Biofilms are structured sessilecommunities of microorganisms attached to biotic or abiotic surfaces and embedded in a self-produced matrix of polymers. In this form, microorganisms may show increased tolerance to stress, antimicrobial drugs and desinfectants, as well as to the immune defences of the host. **Objectives:** The aim of this study was verify the antimicrobial activities of streptomycin and tetracycline against formed biofilms on abiotic surface by aEPEC strains isolated from children with diarrhea. **Methods:** aEPEC pre-formed biofilms on polystyrene plates at 26°C (in LB without NaCl) and at 37°C (in *E. coli* broth) after 24 hours of incubation were treated with streptomycin (100 µg/mL and 500 µg/mL) and tetracycline (50 µg/mL and 250 µg/mL). After 24 hours, the oxidation reduction indicator Alamar blue® (resazurin) was added to the wells and incubated for 1h at 37°C. The reduction relative to untreated controls was determined spectrophotometrically at 550 nm and 595 nm to measure the metabolic activity of biofilms. The biofilm mass was quantified using crystal violet staining assay and this absorbance was measured at 595 nm. **Results and Discussion:** The resazurin assay is simple, sensitive, nontoxic, rapid, high throughput measure of viability after drug treatment. Through this method we could verify that the CB5.10 (low biofilm former strain) and BA4157 (high biofilm former strain) strains presented resistance to streptomycin and in higher degree to the tetracycline, both drugs employed in concentrations above the minimal inhibitory concentration (MIC – 1 µg/mL and 4 µg/mL, respectively), when exposed to these drugs in the biofilm stage. aEPEC biofilms were much less sensitive than their planktonic counterparts, presenting only decrease of viability (44.4 to 90.4% of inhibition), as well as of biofilm mass (12.5 to 89% of inhibition, being the higher inhibition effect in relation to the low biofilm former strain CB5.10 (DO at 595 nm of 0.157 to 0.574), than the high biofilm former strain BA4157 (DO of 0.890 to 1.476). Increasing the concentration of antimicrobial drug, there was a greater inhibition of biofilm formation. The correct administration of drugs to control an infection, mainly in chronic cases may consider the sensibility of biofilm bacteria, due to its capacity to resist to higher antibiotic concentrations than planktonic bacteria, to effectively control the infection.

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5.16 Hfq, a global regulator involved on motility and adhesion in *Escherichia coli* Enteropathogenic

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Introduction: The term atypical EPEC is used to define Enteropathogenic *Escherichia coli* strains that do not harbours the "EPEC adherence factor" (EAF) plasmid. The main feature of EPEC pathogenesis is the formation of the "attaching and effacing" (A/E) lesion in the intestinal epithelium. The genes responsible for this phenotype are contained in a pathogenicity island termed the "Locus of Enterocyte Effacement (LEE). LEE genes can be regulated by Quorum Sensing, a process of bacterial cell-to-cell communication involving the production and detection of extracellular signaling molecules called autoinducers. Quorum Sensing regulation by small RNAs (sRNA) is well established in *Vibrio cholerae.* A 11kDa chaperone termed Hfq also regulates several cellular processes like: decreased growth, stress response and virulence. The role played by Hfq on post transcriptional regulation is related to the pairing of small RNA (sRNA) molecules to near-complementary sequences to its target messenger RNA. **Objectives:** The involvement of Hfq and the sRNA in Enteropathogenic *Escherichia coli* (EPEC) remains unknown. Therefore, this study aims to evaluate the role of the protein Hfq pathogenicity of EPEC E2348/69 typical and atypical EPEC O55:H7. **Methods:** Hfq E:PEC mutants were obtained by allelic recombination using the λ Red recombination system as described by Datsenko and Wanner; and tested for motility (0.3% agar), and adherence to HEp-2 cells. **Results and Discussion:** Mutant strains showed a decrease in motility when related to the wild type strains. On adherence tests, the number of wild type strains adhered to cell surface was higher than in mutant strains. The adhesion to cellular surface is not a process that depends exclusively on LEE. Fimbrial adhesins and flagella are part of this process. The results presented in this study indicate the involvement of Hfq in regulating the LEE region and hence the pathogenesis of EPEC. Hfq is a global regulator described in several processes, which can vary even between samples of the same species.

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5.17 Definition of methodological parameters for Shiga toxin-producing *Escherichia coli*

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Introduction: Globally, each year diarrhea kills around 760,000 children under five and nearly 1.7 billion cases of diarrheal disease occur every year. Shiga toxin-producing *E. coli*, a pathogen recognized as cause of humans disease with varied symptoms, including abdominal cramps and diarrhea which in some cases progress to hemorrhagic colitis and hemolytic uremic syndrome, in which the Shiga toxin production (Stx1 and/or Stx2) are fundamental to virulence mechanisms and the production of these toxins defines the infection caused by STEC.To minimize and control occurrences, the diagnosis is one important tool and immunodiagnostic has several advantages such as high specificity, sensitivity, easy sample preparation and easy implementation.**Objectives:** The aim of this study was to evaluate parameters for production and detection of these toxins in bacterial isolates.**Methods:** In this study, 46 STEC isolates and 45 non–STEC isolates were tested. All isolates were cultivated on *E. coli* broth with 5 ng/mL of ciprofloxacin, wherein the growth was treated or not with Triton X-100, supernatants were separated by centrifugation and tested. In the capture ELISA assay to assess the toxins production, we employed anti-Stx1 or anti-Stx2 IgG enriched fraction and monoclonal antibodies. The cut-off was obtained by ROC (Receiver Operating Characteristics). **Results and Discussion:** In the evaluation of sensitivity, specificity and efficiency the capture ELISA for detection of Stx1 in both treatments was reached 100%, while for the detection of Stx2 in culture treated with Triton X-100, these values were 87%, 83%, and 85%, respectively. However, when the untreated culture these values were 74%, 91% and 83% of sensitivity, specificity and efficiency, respectively. The Triton X-100 treatment allowed better release of Stx2 toxin. In conclusion, the ELISA presented good sensitivity, specificity and efficiency when bacterial cultures were treated with Triton X-100.

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5.18 Characterization of the aggregative adherence pattern of an atypical enteropathogenic *Escherichia coli*

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Introduction: When in contact with cultured epithelial cells some enteropathogenic *Escherichiacoli* (aEPEC) strains express the aggregative adherence (AA), which is a characteristic of enteroaggregative *E. coli* (EAEC). We have previously characterized aEPEC strains of serotype O142:H34 as expressing the AA pattern. **Objectives**: Characterization of the adhesin mediating the AA pattern of O142:H34 aEPEC. **Methods**: The aEPEC strain Ec46/88 was selected for this study. Adherence characteristics and the ability to cause the attaching-and-effacing (A/E) lesion were evaluated in HeLa, HEp-2, Caco-2, T84 and HT29 cells. The search for 23 virulence genes of EAEC was performed by PCR. Plasmid extraction analysis was followed by Southern blot with the *aagA, aggC* and *aggR*sequences of EAEC as probes. Mutagenesis of *aggC* was achieved by a non-polar knockout, and the *aggC* mutant was evaluated for adherence on HEp-2 cells. **Results and Discussion:** The capacity of causing A/E was observed in HeLa and HT29 cells and the AA pattern was observed in all cell lineages evaluated. Only the *aggA*, *aggB*, *aggC* and *aggD* genes were detected, indicating the presence of the AAF/I fimbriae of EAEC. Nucleotide sequencing showed 99% homology with the respective genes of EAEC 17-2, except for the *aggA* gene which demonstrated homology with an *aggA* variant. In aEPEC Ec46/88 the *aggA* and *aggC* genes were located on a 65 kb-plasmid, while hybridization with *aggR* was not detected. Mutation in *aggC* resulted in strong reduction of adherence. Therefore, the AA pattern of aEPEC O142:H34 is mediated by an AAF/I variant not regulated by AggR.

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5.19 *Leptospira interrogans* stimulates and interacts with plasminogen activator.

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Introduction: Pathogenic bacteria utilize a number of mechanisms to cause disease in hosts. Evidences indicate that the pathogens' invasion, dissemination and host tissue damaging depend on proteolytic enzymes both of the invading organism and of the host. Many pathogens express their own proteases or exploit hosts proteases to activate other protease-dependent cascade systems, including coagulation, fibrinolysis, complement activation and phagocytosis. We have described that leptospires capture plasminogen (PLG) on the outer surface, which is converted to active plasmin (PLA) by exogenous activators, endowing the bacteria with proteolytic activity, what favors the penetration and dissemination. **Objectives:** In this work, we aimed to further characterize the interactions of *L. interrogans* with human fibrinolytic system, studying the interactions with PLG activators.**Methods:** We evaluated the ability of leptospires to induce PLG activators in endothelial cells, *in vitro*, and *in vivo* during human leptospirosis.**Results and Discussion:** We show that leptospires induce the expression of PLG activators by human umbilical vein endothelial cells (HUVECs) *in vitro*, what may contribute to enhance the availability of PLG activators *in vivo*. Corroborating this hypothesis, we show that leptospirosis patients have increased levels of circulating urokinase-type and tissue-type PLG activators when compared to normal sera. These levels seems to be especially augmented in the early phase of the disease, and therefore might be a condition important for the initial phase of bacterial infection. We also show that virulent *L. interrogans* express a protein that binds urokinase-type PLG activator, what could favor the conversion to PLA of membrane-bound PLG. The results presented here further characterize the interaction of *Leptospira* with human fibrinolytic system. Taken together, our data suggest the importance of*Leptospira* interaction with PLG and PLG activators to the bacterial dissemination and consequent establishment of the disease.

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6. Biotechnology

6.01 A new method for the Papillomavirus isolation through ultracentrifugation

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Introduction: Papillomaviruses compose a group of oncogenic viruses, affecting different animal species, including human. The difficult of *in vitro* replication and the lack of an adequate viral propagation system in laboratory, justify the need for the development of methods for the virions isolation, which could be employed in immunological challenge. The bovine papillomavirus (BPV) infection causes benign (papillomas) as well as malignant lesions in epithelial and mucosa tissues, causing serious damages to the livestock. Currently 13 types of BPV are described. Besides, the economical losses associated with BPV infections, studies on this virus could serve also as a model for the development of vaccines against human papillomavirus (HPV). **Objectives:** Objectives are the development of a new method for BPV isolation, from bovine cutaneous papillomas, allowing the realization of immunologic challenges for the validation of vaccine products. **Methods:** The cutaneous papillomas were surgically removed.The DNA was extracted and submitted to BPV typification through PCR using the primersδ-ε and ξ, followed by DNA sequencing. Monoviral papillomas were mechanically macerated in an isolation buffer that was clearanced by centrifugation. The supernatant was incubated with 0.25% SDS and 0.01% trypsin for two hours at 37°C and centrifuged at 23,600 rpm in Sorvall swinging rotor AH-629. The pellet was treated with collagenase (2mg/mL) at 37°C. The suspension was centrifuged at 29,100 rpm and the pellet was resuspended in 400μL of resupension buffer, transferred to ultracentrifugation tubes with a solution of CsCl (d=1.3g/mL), which were centrifuged for 24 hours at 34,000rpm in swinging rotor Sorvall AH-650. After centrifugation, BPV virions were collected by suction and stored in PBS at -80ºC. **Results and Discussion:** A purified aliquot was subjected to transmission electron microscopy, revealing isolated virions, indicating the high efficiency of this methodology. This study will allows the use of purified virions in further researches involving the development of animal models for bovine papillomatosis and vaccine testing trough the inoculation of susceptible calves for virus *challenge*. This methodology can be used for the isolation of another papillomaviruses, including HPV, bring new perspectives to the validation of vaccine products.

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6.02 Localization of *Schistosoma mansoni* Venom Allergen-Like 6 transcripts by whole mount *in situ* hybridization (WISH)

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Introduction: Schistosomiasis is considered the most important of the human helminthiases in terms of morbidity and mortality. Genome sequences of the three major schistosome species are now available and research should evolve into its functional genomics phase, aiming to identify the role and importance of schistosome genes that might be targets for drug and vaccine development. The *Schistosomamansoni* Venom Allergen-Like (SmVAL) are members of SCP/TAPS superfamily proteins that are associated with infection processes; however the biological function of this gene family remains unclear. SmVAL6 is an unusual member of this protein family because it contains a SCP/TAPS domain fused to a Micro-Exon Gene (MEG) domain. **Objectives:** One of the first steps towards understanding the function of a gene is to determine its tissue localization. Herein we applied whole-mount *in situ* hybridization (WISH) in adult worms to obtain insights into possible functions of SmVAL6. **Methods:** SmVAL6 digoxigenin-labeled RNA probe was synthesized using T7 RNA polymerase from a cDNA previously cloned in pGEM-T easy vector. Adult worms were incubated with the probe and after several washes, parasites were incubated with anti-digoxigenin alkaline phosphatase conjugated antibody and BM-purple was used as enzyme substrate. **Results and Discussion:** Our previous studies demonstrated that SmVAL6 was transcribed in the oral and ventral suckers of adult male worms. In the current study using a new probe this data was confirmed with a better resolution of the spotted cells in the oral and ventral suckers of male worms, furthermore the tegument cell bodies all over the parasite body seems to be stained. Additionally, it was possible to detect for the first time SmVAL6 transcripts in the anterior and posterior region of adult female worms. In spite of our results the presence of SmVAL6 in the tegument is still a topic of debate in the literature. New experiments of WISH co-localization using a tegument transcript (Sm29) will prove unequivocally whether SmVAL6 is expressed in tegument cell bodies or not. Regarding SmVAL6 function, the suckers are used primarily for attachment and locomotion by the adult male worm, which clasps the female in its gynecophoric canal. The existence of multiple isoforms of SmVAL6 and what benefits would there be to the parasite in maintaining such complex mechanism is still an open question.

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6.03 Production and Quantification of virus pseudoparticles to expression of Rabies Virus Glycoprotein

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Introduction: Rabies is an ancient zoonotic disease and today it still causes more than 55,000 human deaths around the globe each year. The causative agent, rabies virus (RABV), belongs to the genus *Lyssavirus*, family Rhabdoviridae, its genome is a single-strand, negative sense RNA of approximately 12 kb in length and encodes for five proteins in the order of nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (RdRp) or large protein (L). The rabies virus glycoprotein (RVGP) is the only protein exposed in the surface of viral particle and it is mediator in adhesion to cell receptors and to entry in the host cell. The RVGP has been recognized as an antigen able to induce neutralizing antibody of virus, conferring protective immunity against rabies, resulting in the interests of RVGP production in the several systems of known expression. **Objectives:** The aims of this study are establish the system of production of viral pseudoparticles (pp), containing the E1 and E2 proteins of Hepatits C virus, the GAG and POL proteins of murine leukemia virus (MLV) and carrying the RNA coding rabies virus glycoprotein (RVGP). **Methods:** The vectors (pCMVGag/Pol, pCMVE1E2 and pCMVRVGP) were transfected in HEK 293T cells by different methods, electroporation and lipofection. 48h post-transfection the supernatant was collect to obtain the pseudoparticles (pps). The quantification of pps was performed by quantitative PCR (qPCR). Next, we have infected Huh 7.0 cells with different concentrations of the pseudoparticles, in order to measure the RVGP production. **Results and Discussion:** The RVGP presence was confirmed by conventional PCR and the measuring of those pseudoparticles was done through quantitative PCR, and finally we observed that the pseudoparticles were generated correctly, carrying the protein of interest, RVGP.

Supported by FAPESP and CNPq

6.04 Improvement of *Streptococcus pneumoniae* whole cell vaccine production process and its impact on vaccine quality

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Introduction: The currently available pneumococcal vaccines are based on polysaccharides from different serotypes. Unconjugated polysaccharide vaccines are not effective in children less than 2 years old, whereas pneumococcal conjugated vaccines (PCV), which protect small children, are expensive, therefore they are unaffordable for many developing countries. Furthermore, these PCV do not provide coverage for serotypes that are prevalent in many regions of the developing world. Our group have joined efforts to develop a production process of a pneumococcal whole-cell vaccine (WCV), based on a genetically modified unencapsulated pneumococcus strain. This approach represents an alternative low cost, safe and serotype-independent vaccine. **Objectives:** Improve the production process in order to increase the number of WCV doses produced using different fermentation strategies and evaluate these WCV in mouse model.**Methods:** Batch, fed-batch and continuous fermentation with cell recycle were performed at the bench scale (10L) using an animal-free culture medium. Cells were harvested at the highest optical density (OD) of each process and washed 6 times with Ringer's lactate solution with 0.2% glucose by tangential flow microfiltration or by centrifugation. The cell suspension was inactivated with β-propiolactone and stored at -80°C and 4°C. In order to evaluate WCV quality, C57BL/6 mice were immunized with one or two doses of 100 μg protein adsorbed with 200 μg aluminum hydroxide. Twelve days after the last dose, the mice were bled to measure IgG antibody by ELISA and whole-blood samples were stimulated with WCV to evaluate IL-17A production. Mice were challenged by aspiration-sepsis model using 10^6 CFU/animal of an encapsulated virulent strain (WU2). **Results and Discussion:** Higher biomass yields were successfully achieved using the continuous fermentation with cell recycle strategy, which produced 3 times more cells and 2.7 times more estimated number of doses than batch strategy. Our results did not reveal any detectable differences between vaccine preparations, regardless of the fermentation strategy, cell separation method, storage condition and showed similar immunogenicity across all WCV preparations. All WCV produced were able to induce similar levels of IgG between the groups of mice and similar induction of IL-17A *in vitro*. In all cases, protection afforded by the different preparations of WCV was ≥80%, compared to survival of 0-20% in the alum group.These results support the continuous process with cell recycle as strategy for large scale WCV production for human immunization.

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6.05 Purification of Glycosylated Human Prolactin from CHO Cells Adapted to Serum-free Suspension Culture, by Reverse-Phase HPLC

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Introduction: Human prolactin (hPRL) is a 199 amino acid protein hormone (23,000Da) with a wide spectrum of biological activities being, however, best known for its stimulation of lactation and development of the mammary gland. About 10% of hPRL is glycosylated and this form (G-hPRL) is an ideal model for glycosylation studies because, as it contains only one potential asparagine-linked glycosylation site (Asp31-Leu-Ser-Ser), it exhibits a simple type of macroheterogeneity: one protein population with and one without a single N-linked oligosaccharide. **Objectives:** In the present study, G-hPRL obtained from CHO cultured in suspension in spinner flasks was finally purified by reverse-phase HPLC (RP-HPLC). **Methods:** The purification process consists of a first concentration step based on SP-Sepharose fast flow followed by RP HPLC used as a preparative step that can efficiently separate G-hPRL from non-glycosylated hPRL (NG-hPRL). The mass fraction of G-hPRL present on total prolactin was also greatly enriched with the addition of cycloheximide that inhibits protein synthesis by reducing the elongation rate of ribosomes along mRNA, thereby extending the time available for the unique site to become glycosylated. So this drug favours the glycosylation reaction, facilitating its purification. **Results and Discussion:** Our results show that RP HPLC can be an important tool for G-hPRL production, facilitating the purification and characterization of this important isoform of prolactin, whose physiological action is not well known yet.

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6.06 Viral removal validation from antivenom horse immunoglobulin

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Introduction: Viral burden is of great concern when it comes to therapeutic serum products, as immunoglobulins. In order to obtain immunoglobulins free of infective viruses, neutralization steps must be performed and validated. Brazil production relies essentially on equine donors to provide these antibodies, which can carry a large sort of viruses. **Objectives:** Considering the appointments above, this work aimed to develop the method validation for viral removal in the process of immunoglobulin purification from equine serum. **Methods:** The developed method was then used to validate the purification process used in Instituto Butantan and therefore guarantee its safety in this matter. The validation process is achieved by deliberately adding (spiking) a virus material (BVDV Bovine Viral Diarrhea Virus; IBR Infectious Bovine Rhinotracheitis; CAV-1 Canine Adenovirus type 1; CDV Canine Distemper Virus; VSV Vesicular Stomatitis Virus; CCV Canine Corona Virus; BRV Bovine Rotavirus) at various production steps and measuring its removal or inactivation during the subsequent individual step or steps. The purification process was desiccated to identify the steps where viral removal could be happening. The identified steps were set as check points for viral infectivity analysis (TCID50%). Four serum treatments were monitored in the whole process: pepsin and pH at the same time, caprilic acid, heat and phenol treatment. **Results and Discussion:** All steps showed progressive viral neutralization rates, achieving at the end of the process reduction viral particles from the initial burden. This reduction factor (RF in Log_{10}) is considered satisfactory for viral removal, showing that the purification process analyzed is effective and safe.

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6.07 Study of conjugated linoleic acid (CLA) protection in rat liver peroxidation under oxidative stress

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Introduction: Conjugated linoleic fatty acids (CLA) are found naturally in food derived from ruminant animals in its meat and dairy products. To these fatty acids are attibuted several biological properties. **Objetives:** The objective of this study was to determine the CLA effect on liver peroxidation and antioxidant enzymes in rats submitted to oxidative stress by carbon tetrachloride.**Methods:** Eighteen male Wistar rats were divided into three experimental groups: control - H_2O (GI); control + CCL_4 (GII) and CLA 2% (GIII), which were administrated daily by orogastric intubation for twenty one days. Then the animals groups II and III were injected carbon tetrachoride diluted with olive oil, by intraperitoneal route. After twenty four hours all the animals were sacrificed and they livers were evaluated for the peroxidation level by TBARS, and the activity of the hepatic enzymes superoxide dismutase (SOD) and catalase (CAT), as well its gene expression. Statistical analysis was performed by Tukey test. **Results and Discussion:** The results showed significant reduction in the peroxidation level (50%) in the liver of the animals that received CLA 2% (GIII) when compared with the positive control (GII) and similar level with the negative control (GI). The antioxidant enzymes SOD, CAT and GR activies in CLA 2% group were same to the negative control (GI) and higher than the group II submitted to oxidative stress. The results demonstrated the CLA 2% hepatoprotection in the rat oxidative stress conditions.These observations may be useful to understanding the CLA hepatoprotection in oxidative stress.

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6.08 Hemolymph from *Podalia sp* (*Lepdoptera*) prevents apoptosis by enhancing actin and tubulin depolymerization in VERO cells

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Introduction: Apoptosis has a central role in many cellular processes, including embryonic differentiation and development of some diseases like cancer and Alzheimer's. Molecules that interfere in the apoptotic process may be used in the biotechnology industry, especially in the development of products employed in cell culture. Thus, the discovery of new antiapoptotic proteins as well as the control and understanding of their action mechanisms are essential for further progress in this field. **Objectives:** So, the objective of this study, is to identify the potential anti-apoptotic of a protein isolated from hemolymph of larvae of *Podalia sp* (*Lepidoptera: Megalopygidae*). **Methods:** The hemolymph from larvae of *Podalia sp* was collected and the citotoxicity/genotoxicity were evaluated in culture (up to 5%). The anti-apoptotic protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75). The fractions obtained were tested for anti-apoptotic activity in VERO cells. Apoptosis was induced with 25 to 250 µM of Tert-butyl in cells treated and not treated with hemolymph of *Podalia sp*. After 4 hours the cells were incubated with faloidina-FITC to study the cytoskeleton. Microtubules were detected by anti β tubulin and secondary antibody conjugated to Alexa Fluor 488. **Results and Discussion:** Cytotoxicity and genotoxicity of *Podalia sp* hemolymph was evaluated and no adverse effect was observed. This protein was capable to protect VERO cells against death induced by Tert-butyl and was able to avoid the lost of cytoskeleton structure after 24 hours of apoptosis induction. The hemolymph of *Podalia sp* contain components able to inhibit death by apoptosis induced by chemical agents. It was observed that this component can act in cytoskeleton structure, increasing the cell viability acting to maintain the physiological and functional conditions of the cells. So, this product can be of high biotechnological importance to cell culture used for imunotherapic production as viral vaccines and recombinant products.

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6.09 Design of experiments associated to kinetic models for the optimization of *Haemophilus influenzae* type b growth and polysaccharide production

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Introduction:*Haemophilus influenzae* type b infections were a major cause of meningitis in infants worldwide until the introduction of the conjugated vaccine in the 1980's. The antigen used in the vaccine is the polysaccharide, a polymer of ribosyl-ribitol-phosphate (PRP). The presence of a phosphodiester bond in its structure confers a susceptibility to alkaline hydrolysis and, in resemblance to RNA, pH and temperature drive the depolymerization rate. While the optimal conditions for bacterial growth are reported as physiological temperatures and mild alkaline pH's, the stability of the polymer is increased towards low temperatures and acidic milieu. **Objectives:** To evaluate the kinetic parameters of microbial growth as a function of pH and temperature through an experimental design and to optimize the production of PRP. **Methods:** Batch cultures were carried out on a bioreactor with 6 liters of working volume. The process was controlled on pH, temperature and dissolved O_2. An enriched MMP medium was used in order to supply non-limiting carbon and nitrogen sources. pH and temperature were varied in the range of 6.3 - 7.5 and 29 - 37°C in a 2^2 experimental design, with three central points (CP). Dry cell mass concentration was measured by gravimetry; acetic acid by ion exclusion chromatography; and PRP by colorimetry. The data were adjusted to a kinetic model where biomass formation is inhibited by acetate concentration; acetate follows a mixed model of growth-associated and non-associated formation; and PRP formation is growth associated. **Results and Discussion:** In respect to biomass, the maximum specific growth rate increased at lower pH values, what is unexpected for a pathogenic bacterium, while at higher pH the cells were more tolerant to acid inhibition. For acetate, growth-associated formation increased with pH, while the non-associated kept constant. Finally, PRP formation was increased when moving from the acidic to the alkaline region, with the highest values showing at the CP (33°C; pH 6.9). In conclusion, the results demonstrated that pH interferes strongly, modifying the growth profile and PRP formation inversely; acid production and inhibition are also changed by pH. Regarding temperature, there seems to be no effect on the parameters but the expected slower metabolism. The maximum productivity of PRP was found at the CP, achieving up to 96 mg/L/h, about 15% more than the conditions reported in the literature. Simulations of the model presented here is useful in predicting the optimal conditions for PRP production and in designing new processes, such as fed-batch and continuous cultures.

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6.10 Antiviral effects of *Scaptotrigona postica propolis*

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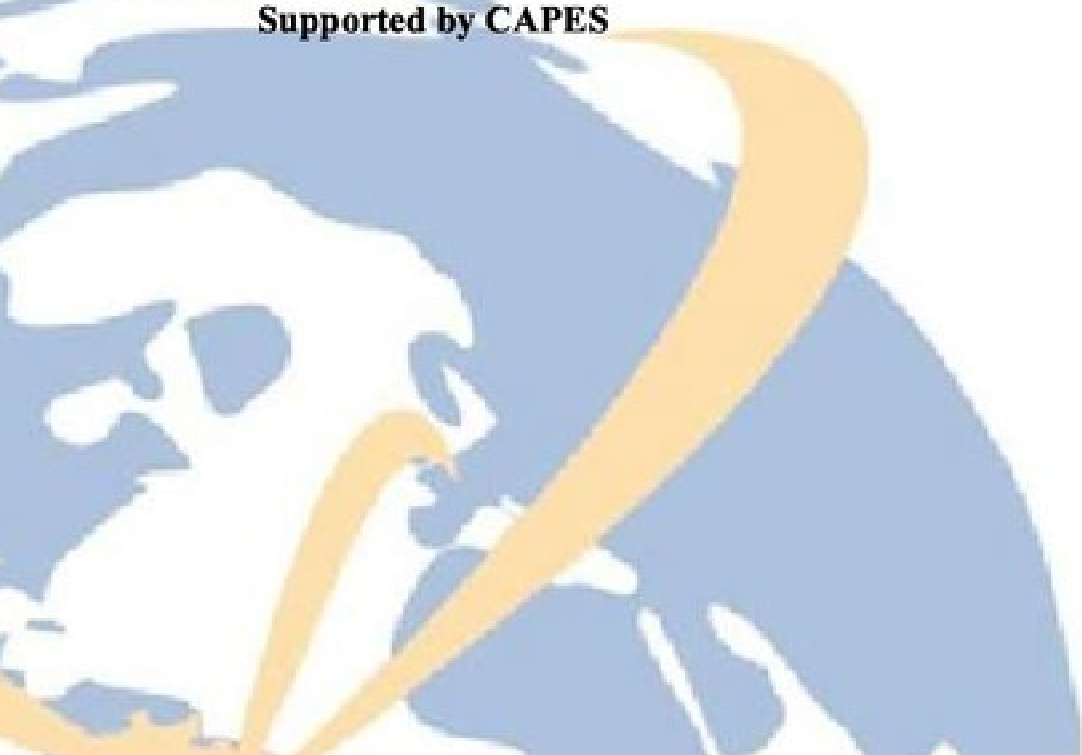
Introduction: Studies about viral infections have a great importance in human and veterinary health in actual days, and the number of medications available to treatment these diseases is very reduced, making the search for antiviral molecules an important focus for scientific research. Propolis is a bee material manufactured by the mix of exudate of plants, saliva and bee wax. This product is used to seal the hive and involve dead invaders. Their chemical profile is very variable, and depending on the geographic origin and plant conditions of growth. The use of propolis (bee-glue) for various purposes has reports at before Christ. In Egypt, propolis was used in the preservation of bodies, performing a function of balsam, and its use persists to today in folk medicine to treat various pathologies, being widely used around the world. It is known that propolis of *Apis mellifera* has compounds with antiviral activity on virus like Influenza A and B, Vaccinia virus, Hepatitis virus, HIV, Herpes virus, HIV and Poliovirus. In Brazil exists a subfamily of bees named Meliponinae, the stingless bees mixing the resins collected of plants with wax and ground, producing a different type of propolis, named geopropolis, but not all bees of this family produce this type of propolis, like *Scaptotrigona postica*. The biological activities of *Scaptotrigona postica* propolis remain unknown, the little information about this product is concentrated in antibacterial and anti fungi actions, but don't has related of antiviral action. **Objectives**: Purify, isolate, and characterize substances with antiviral activity of propolis from *Scaptotrigona postica*. **Methods**: The propolis collected in Barra do Corda, city of Maranhão state, was partitioned with hexane, ethyl acetate and a solution of water/methanol (1:1). The propolis crude, as well its purified fractions, were tested by viral titer reduction technique, and determination of viral mRNA in microplate with 96 wells, against measles, picornavirus, influenza virus and rubela virus. **Results and Discussion:** Experiments with the purified fraction led to a 64-fold reduction of picornavirus production, 32-fold reduction in influenza virus production and 8-fold reduction of measles virus. Assays using RT-PCR, to determine viral mRNA present in the treated and infected cells, also was performed. The purified antiviral fraction was able to reduce at 10^3 times the replication of rubella virus. At the moment, we are performed the optimization of the purification process.

Supported by CAPES



6.11 Development of a lateral flow immunoassay for diagnostic of *Bordetella pertussis*

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Introduction: *Bordetella pertussis* is a highly infectious bacteria, responsible for whooping cough, prevalent disease worldwide, reaching all ages but particularly severe in child less than six months of age. The real incidence of the disease is not very clear, due to the number of related cases classified only as suspicious and the absence of a sensitive and rapid test for diagnostic. The lateral flow immunochromatographic assays, also known as "dipsticks", are alternatives for rapid identification assessments and epidemiological control. Using colored particles, they are based on the immunocapture principle in a solid phase, which two antibodies bind to different epitopes. **Objectives:** This study has the objective of developing a rapid and sensitive lateral flow immunoassay for *Bordetella pertussis* to enable a quick and effective diagnostic tool. **Methods:** Gold colloidal particles (Sigma) were conjugated to IgG from anti-Plow, a polyclonal serum against a whole cell pertussis vaccine with low content of LPS (Plow), in development in our laboratory. This conjugate is used as a detector antibody, immobilized in a glass fiber in one of the extremities of the dipstick and bind to the antigen sample, a whole cell pertussis vaccine from Instituto Butantan (WCV). The immunocomplex run through the solid phase, a nitrocellulose membrane (MN) and is cached in the test line containing the capture antibody, a polyclonal serum anti-pertussis toxin (αPTM-6). The excess of antibody-antigen complex is captured by the control line by anti-mouse IgG. Two visible lines in the dipstick (control e test) are considered a positive result and only the control line, negative. **Results and Discussion:** The dipstick manufactured with anti-Plow conjugated with gold colloidal particles and αPTM-6 as capture exhibited a detection limit of $3{,}5 \times 10^7$ cells/sample of 350µl of WCV. PBS was used as a negative sample control, presenting only the control line, and allowing for the specificity of the test. The rapidness in the visualization of the results (within 20 minutes) suggests this assay as a possible alternative to traditional methods for diagnostic of *B. pertussis*.

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6.12 Characterization of the interaction between fibrinogen and the leptospiral protein Lsa33

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Introduction: Leptospirosis is a zoonosis of global importance that is being considered an emerging infectious disease. Studies have been conducted to characterize a novel antigen for its ability to interact with the plasma component fibrinogen (Fg). The recombinant protein Lsa33 (Leptospiral surface adhesin of 33KDa) has been shown to interact with laminin, plasminogen and C4bp. **Objectives:** We have now evaluated the ability of this protein to interact with Fg. **Methods:** The capacity of Lsa33 to mediate attachment to Fg was performed by ELISA. The binding was assessed by different conditions: increasing protein concentration, using specific antiserum against the protein, previous denaturing the recombinant protein, and using metaperiodate oxidized- Fg. The fibrin clot formation inhibition assay by Lsa33-bound to Fg and the effect Lsa33 on the binding of live leptospires to Fg was also evaluated. **Results and Discussion:** Lsa33 interacts with Fg in a dose-dependent and saturable fashion. The binding was totally inhibited by antibody-blocked protein, and partially inhibited by denatured protein, suggesting that binding epitopes were partially exposed. There was no reduction in the interaction of Lsa33 to oxidized Fg, implying that carbohydrate moieties are not involved in the interaction. The binding of Lsa33 with Fg resulted in inhibition of fibrin clot formation by thrombin-catalyzed reaction. The Lsa33 attachment to Fg caused significant reduction in the number of leptospires adhering to Fg. We believe that this multifunctional protein has the potential to participate in the interaction with components of the coagulation cascade and fibrinolytc system that might help bacterial disseminate through the host.

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6.13 Functional and Immunological Characterization of Two Recombinant Leptospiral Proteins

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Introduction: Leptospirosis is been considered an important re-emerging infectious disease caused by pathogenic *Leptospira* spp.. Available vaccines based on inactivated whole cell of leptospiras do not induce long-term protection against infection and do not provide cross-protective immunity. Surface exposed proteins are potential targets for inducing immune responses during infection and may also mediate the initial adhesion process to host cells. **Objectives:** To evaluate the immunological response of two recombinant proteins, rLIC10645 and rLIC10731 of *L. interrogans,* predicted to be outer membrane proteins (OMPs), and their capacity to adhere to serum proteins and extracellular matrix components. **Methods**: BALB/c mice were immunized subcutaneously, bled from the retro-orbital plexus and the antibody response evaluated by ELISA and Western blotting. Splenocytes were isolated for evaluation of lymphocyte proliferation and cytokine profiles. The attachment of these proteins to macromolecules was evaluated by ELISA. Reactivity with antibodies present in serum of confirmed leptospirosis samples was also performed by ELISA. **Results and Discussion:** Proteins promoted high antibody levels in immunized mice. rLIC10731 showed the highest proliferative rate and a significant increased level of IL-10, IFN-gamma and TNF-alpha. Both proteins were recognized by serum from leptospirosis patients; also, they displayed attachment to plasminogen and laminin, being named, thus, Lsa44 and Lsa45 (Leptospiral surface adhesin plus molecular mass) for rLIC10645 and rLIC10731, respectively. These data suggest the involvement of these proteins in the leptospiral adhesion and infection process.

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6.14 Tissue expression patterns of *Schistosoma mansoni* Venom Allergen-Like proteins 1, 13, 14 and 24 by Whole Mount *In Situ* Hybridization (WISH) in intramammalian stages

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Introduction: The *S. mansoni* Venom Allergen-Like proteins (SmVALs) are members of the SCP/TAPS (Sperm-coating protein/Tpx-1/Ag5/PR-1/Sc7) protein super family, which may be important in host-pathogen interactions. It is a large gene family with 29 members, divided in two groups (group 1 secreted, maybe acting as immunomodulators during parasite migration; and group 2, likely to be involved in intracellular interactions). Recently, a series of transcriptome, proteomic and microarray studies highlighted SmVAL members as potential targets for immune intervention; however the biological function of this gene family remains unclear. **Objectives:** One of the first steps towards understanding the function of a gene is to determine its tissue localization. Herein we applied whole-mount *in situ* hybridization (WISH) to obtain insights into possible functions of four SmVAL members, namely, SmVAL1, 13, 14 and 24. Additionally, we aimed to express and purify the respective recombinant proteins to evaluate them as vaccines candidates in the murine model for schistosomiasis. **Methods:** SmVALs digoxigenin-labeled RNA probes were synthesized using T7 RNA polymerase from cDNAs previously cloned in pGEM-T easy vector. Germballs and cercariae were incubated with SmVAL1 and 24 probes while 7 week adult worms were incubated with SmVAL13 and 14 probes. After several washes, parasites were incubated with anti-digoxigenin antibody-alkaline phosphatase conjugated and BM-purple was used as enzyme substrate. **Results and Discussion:** SmVAL1 and 24 transcripts were localized in germball's pre-acetabular glands and in cercariae's head gland, while SmVAL13 and 14 transcripts were localized in adult male's anterior esophagus. These results suggest that SmVAL1 and 24 probably act in two different moments of parasite migration. First during skin penetration, when the content of pre-acetabular glands is released and second, during a blood vessel invasion, when it is believed that the head gland content takes part. On the other hand, the expression of SmVAL13 and 14 in the anterior esophagus of adult worms suggested a role of these genes in the blood feeding process. In this context, a rational to be tested for a vaccine design would be to block parasite migration and the feeding process with a vaccine cocktail of SmVALs.

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6.15 LexA1 Regulon of *Leptospira interrogans* serovar Copenhageni

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Introduction: Human leptospirosis is a disease caused by *Leptospira interrogans* and mainly transmitted by water contaminated with rodents' urine. The ability to infect a new host after weeks in water or mud indicates that this bacterium has a complex system of detection and response to different environmental stresses. The SOS system is one of the stress responses usually linked to increase in virulence in pathogenic bacteria. The system is triggered by DNA damage, and the transcription of the involved genes is controlled by the LexA repressor. Unlike the majority of organisms, the serovar Copenhageni harbors two LexA: *lexA1* and *lexA2*. **Objectives:** The aim of this study was to identify the targets of LexA1, which is probably the main SOS repressor, and the DNA motif responsible for LexA1 recognition, using chromatin immunoprecipitation coupled with massive parallel sequencing. **Methods:** Cultures were cross-linked with formaldehyde, lysed and the genomic DNA was sheared by sonication. Lysates were incubated with anti-LexA1 serum and complexes of LexA1-DNA were precipitated using Protein G-Sepharose. The cross-links were reversed and the purified DNA was submitted to sequencing. The reads were aligned to the reference genome using Bowtie2 and enriched regions were identified by the peak calling algorithms SISSRS and CisGenome. *De novo* motif discovery was carried out using MEME. **Results and Discussion:** The immunoprecipitations resulted in 3-7 fold enrichment of the *recA* promoter (a known LexA1 target) relative to the coding region of *flaB*, as assessed by quantitative PCR. Peak calling in the sequencing data identified 40 enriched regions with $P_{value} > 0.001$. The number of possible targets was trimmed down to 28 by considering only peaks with more than two fold enrichment above background. Among the identified genes, there were several DNA repair and metabolism related genes (as *recA*, *recN*, *lexA1*, *lexA2*, *dinP*, helicases), a couple of possible transcriptional regulators (containing helix-turn-helix domains), flagella constituents (*fliF, fliG, fliH*) and enzymes involved in carbohydrates and fatty acids metabolism. A MEME search throughout the peaks' sequences generated a palindromic motif, in a 7+2+7 fashion (each half containing seven positions with a two-nucleotide spacer). Our results show a complex network of response, involving several aspects of the cell biology, but focusing in DNA-related processes. The LexA1 binding motif is surprisingly degenerated, with the smaller spacer among the published LexA motives for other bacteria.

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6.16 Evaluation of the relationship between agitation, dissolved oxygen and concentration of *Neisseria lactamica* OMV in batch cultivation

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Introduction: Mucosal delivery vaccines have been an advantageous alternative of immunization. Firstly, due to its simplified administration and secondly, because of the potential of conferring protection at the natural site of infection for many pathogens. That is the case of the pathogenic bacterium *Neisseria meningitidis*, commonly known as meningococco. Outer membrane vesicles (OMV) from a commensal microorganism, *Neisseria lactamica*, induce antibodies that cross-react with meningococco; in addition, that OMV can act as an effective intranasal adjuvant. **Objectives:** Evaluate the agitation rate and the percentage of dissolved oxygen over 15h *N. lactamica* batch cultivation and establish the relationship of these two factors with the highest concentration of OMV released. **Methods:** The batch process conditions comprised: 5L bioreactor, 36 °C, 0.2 bar, overlay air flow rate of 1L/min, agitation varying from 250 to 850 rpm and dissolved oxygen at 30%. Experiments were carried out using Catlin medium with threefold of its concentration of lactate and double of its concentration of amino acids plus 2g/L of yeast extract. In a first condition, a pulse of lactate was done at the eighth hour of cultivation and in another one, a pulse of lactate plus amino acid was accomplished at the same time. All essays were realized in duplicate by 15h cultivation. Samples were collected from cultivation hourly in order to measure its biomass and protein. Bacterial growth was estimated by optical density (OD_{540}nm); biomass was measured by dry weight per liter (g/L) and the dosage of protein was established by Lowry's method (mg/L). **Results and Discussion:** The results are represented by the average of duplicates. In the first condition, the maximum bacterial growth and dry weight observed was 6.87 (OD_{540}nm) and 3.48g/L, both at the 13th hour of cultivation and the highest yield of OMV was approximately 308 mg/L at 15th hour. In the second, the best result for bacterial growth and dry weight was 6.72 (OD_{540}nm) and 3.53g/L at the 15th and 14th hour, respectively. The peak of OMV's yield was around 349 mg/L at hour 13. The results indicate that the peak of OMV released is not completely associated to *N. lactamica* growth. Interestingly, the highest concentration of OMV overlapped the time of cultivation when agitation rate decreased and the percentage of dissolved oxygen increased, implying cellular stress followed by death. These two parameters will be adopted going forward as indicators of the highest yield of OMV during batch cultivations, which aims enhance its productivity in order to evaluate its mucosal adjuvant function.

Supported by CAPES

6.17 Characterization of LIC10377 coding sequence of *Leptospira interrogans* by bioinformatics

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Introduction: Leptospirosis is an important widely spread zoonosis caused by pathogenic *Leptospira* spp. The disease is transmitted from reservoir hosts to humans through contaminated water and soil. In Brazil the predominant agent of leptospirosis is *L. interrogans* serovar Copenhageni. Currently, available commercial vaccine based on inactivated cells of leptospira does not induce immunological memory and only protects against serovars included in the preparation. For cross protective immunity, surface exposed and extracellular proteins are potential targets to initiate immune response during infection cases. The improvement of biotechnology, as databases and complete genome sequences allow us to study and characterize news genes. This study refers to the gene LIC10377, genome annotated as hypothetical lipoprotein. **Objectives:** Tocharacterize *in silico* the protein encoded by LIC10377 gene for accessing its potential as vaccine candidate. **Methods:** The similarity and conservation of the protein sequence with the available sequences on the database from others serovars and species were evaluated by BLAST software. Multiple sequence alignments were performed by CLUSTALW software. CELLO and SOSUI gram N webservers were used to evaluate the cellular location of the predicted coding sequence. The presence of signal peptide, lipidation and cleavage site predictions was performed by LipoP program; SignalP webserver was also employed for peptide signal identification. **Results and Discussion:** The LIC10377 gene is genome annotated as a probable lipoprotein of estimated 27.5 kDa molecular mass, conflicting with the results obtained by LipoP software that does not predict the presence of lipobox. The BLAST analysis showed that this sequence is presented with high similarity in pathogenic strains, an important factor for wide-ranging immunity protection. The cellular location prediction showed that this putative protein is probably extracellular or periplasmatic. The predicted cellular location, allied with sequence conservation, makes this protein a potential candidate for recombinant vaccine.

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6.18 Expression of heterologous antigen in recombinant BCG through genetic engineering

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Introduction: The Bacille Calmette-Guérin or BCG promotes an intense immune response that is the baseline for using BCG as a live recombinant vector system to express antigens of different pathogens. By transforming the bacillus with mycobacterial shuttle-plasmids we can drive the expression of a wide range of viral, bacterial and parasitic antigens. However, the level of gene expression obtained can be variable due to codon usage preferences among organisms, plasmid copy number or stability and most importantly the promoter strength. **Objectives:** Generate recombinant BCG strains presenting different promoter strengths and evaluate their immunogenicity. **Methods:** The mutation tool used was based on error-prone PCR using modified dNTPs. The original pX promoter in the pX-GFP plasmid was removed and replaced with the mutated versions of pX upstream the GFP gene sequence. This library of plasmids was then used to transform *M. smegmatis* and *M. bovis* BCG and analyzed with respect to their fluorescence by flow cytometry and confocal microscopy, which in turn represent the promoter strength. These promoters were then used to direct the expression of codon optimized Sm29 antigen in recombinant BCG and the immune response induced against the rBCG strain expressing Sm29 was assessed in immunized mice. **Results and Discussion:** Promoter mutants showed a specific strength regarding their capacity to generate GFP and therefore fluorescence both in *M. smegmatis* and *M. bovis* BCG. The promoter capable of inducing higher fluorescence showed higher levels of Sm29 antigen production and the weaker showed a faint Sm29 production. Mice immunized with rBCG expressing high amounts of Sm29 antigen exhibit a specific immune response of cytokines and but antibodies. Since the expression level is carefully regulated to avoid wasting energy with unnecessary metabolites, defining the heterologous expression in this condition is perhaps the most important component of the regulatory control process. We propose here a strategy to engineer promoters to control the gene expression in mycobacteria, thus improving its application as a recombinant delivery system.

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6.19 Development of the purification process of the hybrid: pneumococcal surface protein A and genetically detoxified pneumolysin

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Introduction:*Streptococcus pneumoniae* is cause of various diseases such as pneumonia, otitis, sinusitis, meningitis and sepsis. The vaccines currently on the market are based on the capsular polysaccharide (PS), free or conjugated to a protein, but their high production cost does not allow their distribution to the entire affected population. Moreover, the vaccines available in Brazil do not cover all cases of invasive pneumococcal disease in the country. In order to reduce the cost and increase the coverage of the pneumococcal vaccine, the production of a conjugate vaccine including PS of prevalent serotypes linked to pneumococcal proteins has been proposed. Among the proteins studied, the pneumococcal surface protein A (PspA) and pneumolysin (Ply) showed the most promising results in sepsis model and the combined use of these two proteins showed the greatest protective potential against systemic challenge in mice. Due to its toxicity, Ply was genetically detoxificated (PdT) to be included in the vaccine. **Objectives:** To develop a purification process for the recombinant hybrid PspA94-PdT, expressed in *E. coli* with M15 with N-terminal histidine tag. **Methods:** The lysis was performed in continuous high pressure homogenizer with temperature and pressure control. The optimal time of the lysis was determined by SDS-PAGE and analysis of the cell density at 600nm and absorbance of lysis supernatants at 280nm. The clarification was performed by precipitation of contaminants with the cationic detergent cetyltrimethylammonium bromide (CTAB) and centrifugation. The optimal CTAB concentration added to the homogenate was also determined. The homogenate was then centrifuged at 16.696g and 4ºC for 2 h. The clarified material was applied to an ion exchange chromatography. The protein concentration was determined by Lowry and the purity by SDS-PAGE and densitometry. **Results and Discussion:** The optimal lysis time was 8 min, at 4ºC and 500 bar. The optimal detergent concentration, which precipitated contaminants with no significant loss of the target protein, was 0.1%. After clarification, the purity increased from 25.0% to 40.6% with 72.3% of yield. The target protein was eluted from the ion exchange chromatographywith 200mM NaCl with 54.0% of purity and 48.7% of yield. In order to achieve a higher yield and purity, required to the conjugation process, it will be necessary to continue the purification with other chromatographic steps. Metal affinity, hydrophobic interaction and mix mode chromatography will be tested. The best sequence will be repeated in order to evaluate the robustness and costs of the production process developed.

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6.20 Rabies virus replication in different bioreactors

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Introduction: The scaling up of virus production process in bioreactor involves different challenges like to overcome the problem of homogeneous mixing without mechanical damage to animal cells, but providing efficient meeting between cells and virus. **Objectives:** The objective of this study was to compare the rabies virus replication in two bioreactors, one of 30 L (BioFlow 4500, NBS) and other of 150L (BioFlo PRO Industrial, NBS). These bioreactors present different agitation systems: while the 30 L has a "cell lift impeller", the industrial, one STR, has pitched blade impellers. **Methods:** Vero cells added to solid microcarriers, Cytodex 1 (2g/L), infected with PV rabies virus (MOI 0.02) were cultivated in serum-free medium VP SFM AGT in the bioreactors. Were realized 44 cycles (20 in the bioreactor of 30 L and 24 in the 150 L) classified in three different initial cellular concentration by microcarrier: low (9-12 cells), intermediate (16-19 cells) and high (24-39 cells). The harvests of supernatants of these cultures started on days 2 or 3. The samples of the infected culture were taken every day to determine virus titers (FFD_{50}/ml) and cell concentration. **Results and Discussion:** The rabies virus titers found in the harvest obtained in the cycles of bioreactor 30L were $10^{5.1}$, $10^{5.3}$ and $10^{5.0}$ FFD_{50}/ml to low, intermediate and high cellular concentration; for the bioreactor of 150L were $10^{4.5}$, $10^{4.6}$ and $10^{4.3}$ FFD_{50}/ml respectively. The results show that the system of agitation of the bioreactor had a high influence in the infection the cells by the rabies virus. The "cell lift impeller" used to agitation of the culture is more efficient to rabies production in bioreactor.

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6.21 Production of viral particle for the NS3-HCV expression in mammalian cells

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Introduction: One of the major health problems in the world is hepatitis caused by hepatitis C virus (HCV), given that the current treatment provides satisfactory immune response only to some patients. Multiple approaches have been tested in order to develop a vaccine to prevent hepatitis C. DNA vaccines and virus-like particles, carrying portions of HCV genome are under development. **Objectives:** Within this perspective, our work aims to produce two viral pseudoparticles. One is based on the Semliki Forest Virus (SFV-HCV) system and the other on chimeric particles of Murine Leukemia Virus (MLV) and HCV (ppHCV). Both systems carry the gene for a portion of the nonstructural protein 3 (NS3p) of HCV. These pseudoparticles have been produced and used for "infection" of mammalian cells in minimal medium (supplemented with 10% FBS) or serum free media (SFM). **Methods:** For the synthesis of ppHCV-NS3p particles three vectors: pCMVGAGPOL, pCMVE1E2 (kindly supplied by Cossette) and pTGNS3p1a were transfected by electroporation of HEK293T cells. And for the synthesis of SFV-NS3 particles two vectors: pSFVHelper (kindly supplied by Renaud Wagner) and pSFVNS3p1a were *in vitro* transcripted and transfected by electroporation of BKH-21 cells. **Results and Discussion:** Our results show the production of 2.5E4 particles of SFV-NS3p per microliter and 4E2 particles of ppHCV-NS3p per microliter. In addition to the production of the viral pseudoparticles, we performed the adaptation of human hepatoma cells (Huh7), in SFM (CHO®, VP® and PRO293®). Cell infection with the produced pseudoparticles is under investigation in order to compare the NS3p expression in different systems and conditions. The protein production is expected to be higher in cells grown in SFM that can promote a higher entry of particles in cells.

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6.22 Transcutaneous immunization of mice with recombinant protein LipL32 of Leptospira

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Introduction: Leptospirosis is caused by gram-negative spirochetes and is acquired through contact with the urine of infected animals and damaged skin or mucosa, and currently there are over 500,000 cases of leptospirosis worldwide. Transcutaneous immunization (TCI) is an alternative to topical vaccine administration, with lower degree of invasiveness and does not require trained personnel. The use of vaccines against Leptospira using the TCI would reproduce the natural route of infection. **Objectives:** To evaluate, in mice, the induction of humoral immune response after TCI with recombinant LipL32 of leptospira. **Methods:** Balb/c mice (18-22g) were immunized with LipL32 by transcutaneous route (tc) after shaving the abdominal region with a razor. Three types of emollients were used as pre-treatment for 15 minutes before application of LipL32 or by mixing it with LipL32: (1) Surfactants: (i) a pig lung surfactant produced by Instituto Butantan (IB) (surf But); (ii) a synthetic surfactant kindly gift by Dr. José Gregório C. Gomes from University of São Paulo (surf USP) and (iii) a commercial surfactant (surf com); (2) Monophosphoril lipid A from *B. pertussis*, produced by IB (MPL) and (3) Polyethylene glycol (Sigma) (PEG). Groups of 3 mice received the vaccine by tc route, in 3doses of 10 µg/20µl, within 7 days intervals. 50 days after the third dose, Lip32 was administered intradermally without emollient (ID) (1µg/200µl) and 36 days later by tc route (1µg/20µl). A control group received PBS under the same schedule and another, only LipL32 by ID (1 µg/200µl) and 36 days later 1 µg/20µl by tc route. The total IgG against LipL32 was measured by ELISA on individual sera of test and PBS groups 15 days after the third tc dose, 7 and 22 days after ID, and 15 days after the tc 1 µg/dose. The LipL32 ID control group was bleed under the same bleeding schedule, after the ID immunization. **Results and Discussion:** The immunization with Lip32 by tc route induced merely detectable serum IgG antibodies, 15 days after the last primary immunization. The ID sub immunizing dose of Lip32, that not induced detectable IgG titers in the control ID group, was capable of induce memory antibody response in the test groups, more than 70 days after the tc primary immunization, with enhanced titers in average 435 times after a tc sub immunizing dose of Lip32, 36 days after the ID dose. The TCI with LipL32 mixed with the emollient apparently induced better results when compared with the pre-treated groups. Our data suggest that all test groups showed to be primed by the TCI with Lip32 and that a memory antibody response could be achieved, confirming the effectiveness of this approach for LipL32 entrance.

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6.23 Synthesis and purification of the PS1-PspA conjugate

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Introduction:*Streptococcus pneumoniae* is a pathogenic encapsulated bacterium that causes infectious diseases, one of the main cause of death between young children. The antigen of vaccines against *S. pneumoniae* are capsular polysaccharide (PS) free or conjugated to a carrier protein. The advantage of a conjugated vaccine is to change the PS from a T-cell independent antigen to a T-cell dependent antigen causing generation of memory cells. **Objective:** Synthesis and improvement of the purification process of the capsular polysaccharide of *Streptococcus pneumoniae* serotype 1 (PS1) and pneumococcal surface protein A (PspA) conjugate. **Methods:** The methodology consisted of two steps: 1) Conjugation. The conjugate was obtained in three steps: hydrolysis of the polysaccharide, carboxamide formation (PS1-AH) andconjugation reaction between PS1-AH (15 mg) and PspA (15 mg). 2) Purification Step. To study the purification of the conjugates was tested two types of chromatography: hydrophobic interaction and size exclusion chromatography. The reaction mixture was purified by hydrophobic interaction chromatography using Phenyl Sepharose 6 Fast Flow eluted in a gradient of 2M - 0M $(NH_4)_2SO_4$ at flow of 2.5 ml/min. The purification by size exclusion chromatography was performed in Sephacryl S-400 or Sephacryl S-300 and eluted with 0.15M NaCl, 0.05M Na_2HPO_4, pH 7.0 at flow rate of 2.5 ml/min. Polysaccharide and protein contents were measured by phenol-sulfuric and bicinchoninic acid (BCA) methods, respectively. **Results and Discussion:**The average molecular weight of the PS1 after hydrolysis decreased from 1,000 kDa to about 36 kDa. The carboxamide formation introduced 3 groups NH_2 per molecule of PS1. The group NH_2 of the PS1-AH reacted with the carboxyl group of PspA. The purification of the conjugates by hydrophobic interaction chromatography or size exclusion chromatography with Sephacryl S-400 resin revealed the presence of conjugate PS1-PspA. However, in both cases, the conjugate was not accordingly purified. The purification by hydrophobic interaction chromatography showed to be not adequate because the hydrophobic characteristics of conjugate were not so different from the characteristics of unconjugated PS1. In the size exclusion chromatography using S-400 the resolution of purification was also not good. Nevertheless, the size exclusion chromatography by Sephacryl S-300 resin seemed to be more efficient.

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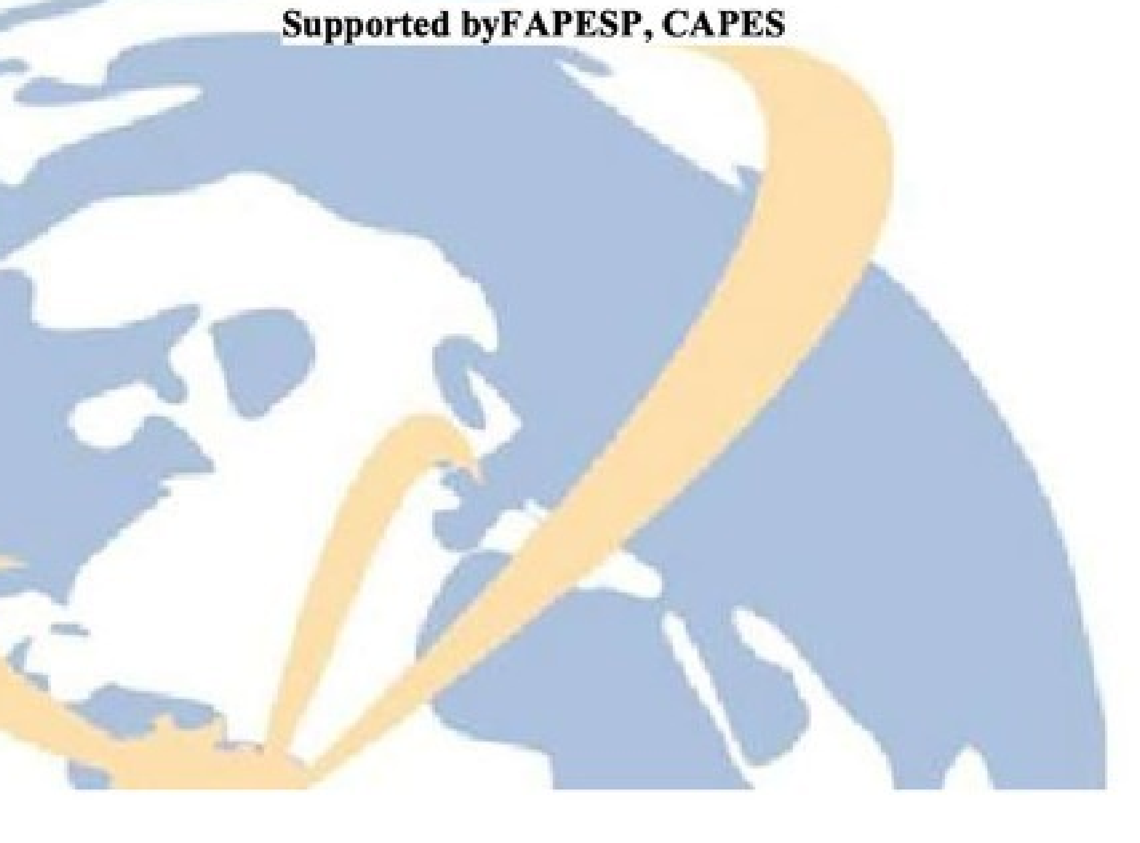

6.24 Partial Results of the Construction of cDNA library of *Megalopyge albicollis*

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Introduction: For more than 350 million years, insects live and survive in almost every ecosystem on the planet, what have resulted in the development of protection and defense mechanisms against adverse situation. This feature has stimulated research into new agents with pharmacological and biotechnology potential in the class of arthropods.
Recently, we have identified and isolated proteins of pharmacological and biotechnological interest in the hemolymph of caterpillars from family Saturnidae (*Lonomia obliqua*). Two proteins have been further characterizes: one with antiapoptotic activity and other with an antiviral action. **Objectives:** The main objective of this project is to build, characterize and compare the transcripts generated by the construction of a cDNA library of the integument of caterpillars*Megalopyge albicolis* (Megalopygidae family). **Methods:** The mRNA was isolated using the Dynabeads mRNA Direct kit (Invitrogen) and quantified with the RiboGreen RNA Reagent (Invitrogen). Subsequently, a cDNA library was produced and sequenced with a 454 GS-Junior machine (Roche). These procedures were repeated for two different tissue samples, originated from two different animals. **Results and Discussion:** As a result, we obtained 38,2456 reads for the first tissue cDNA sequencing and 138,177 for the other one, with an average size of 240,66 and 439,10 bases, respectively. After the elimination of small and low quality reads, the poly A tails were trimmed off, resulting in 165,187 reads with an average size of 398,87 bases. These sequences were grouped according to their similarity using the CLC-Genomics program, resulting in 2,519 Contigs (using 118,777 reads), while the remaining 46,410 reads were classified as singlets or singletons. The more abundant transcripts are mainly related to proteins involved in catalysis, transport and binding. We also detected a high expression of the gene encoding for the protein arylphorin, with is involved in storage and in developmental and metamorphosis processes. Other frequent transcripts are also related to storage (vitellogenin) and to reactive oxygen species detoxification (catalase).These proteins need be further studied to better characterize their biological role, as well as to determinate the biotechnological potential of molecules produced by *M. albicolis*.

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6.25 Comparative analysis of rabies virus glycoprotein expression in two culture media

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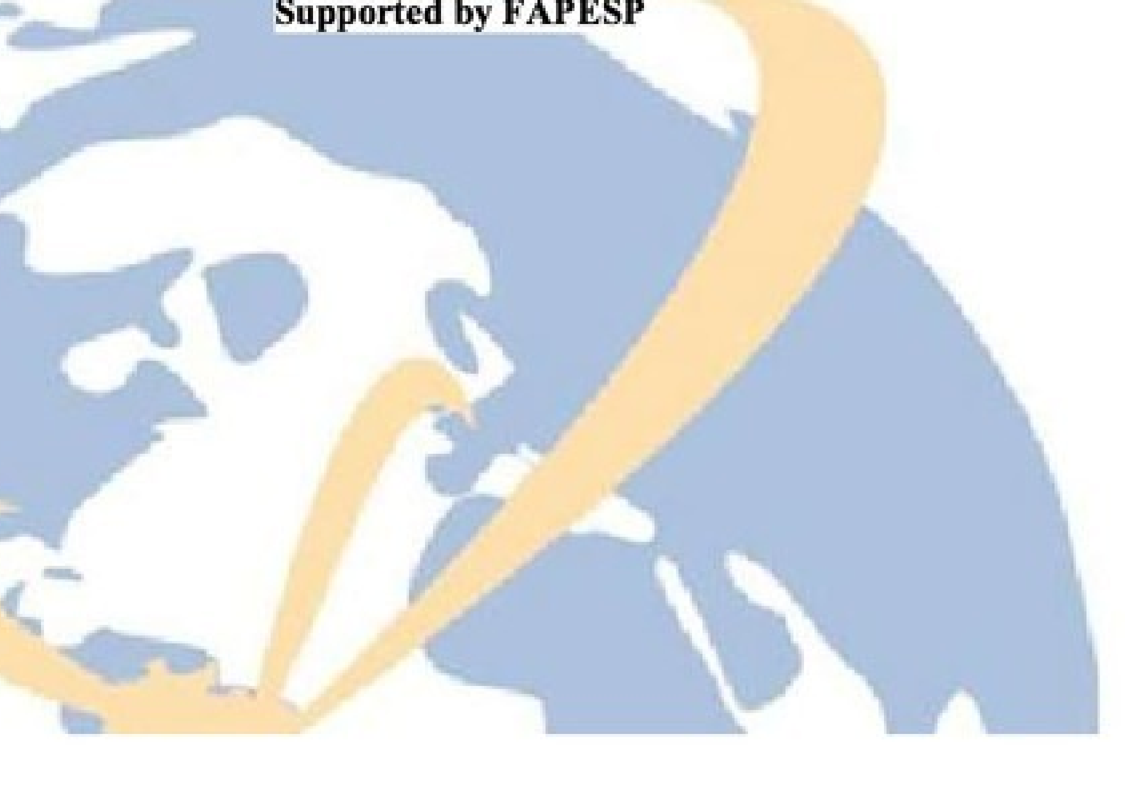
Introduction: Insect cells from Drosophila melanogaster (Schneider, S2 cells) have been used for the expression of rabies virus glycoprotein (RVGP). Studies of RVGP quality and productivity of the system are being performed. As recombinant glycoproteins are subject of different post-translational modifications depending on the metabolic state of culture, it is of interest to evaluate the differences between the RVGP produced by the same system in different culture media. **Objectives:** To compare the productivity and antigenic properties of the RVGP produced by S2MtRVGP-His cells cultivated in two different culture media. **Methods:** S2MtRVGP-His cells, initially growing in SF900II, were progressively adapted to IPL41culture medium. Adaptation was performed with different medium composition and cultivation times, beginning with 30% SF900II and 70% IPL41, for one week. As no visible changes in viability were noticed, the percentage of IPL41 was increased by 20%. Adaptation was taken over 10 weeks changing media concentrations (IPL41medium concentrations were 30%, 50%, 65%, 80%, 90%, 96%, 98% and finally 100%). After adaptation, cells were submitted to selective pressure with hygromycin (600 µg / mL). S2MtRVGP-His were cultivated twice in each SF900 and IPL41 media for study of growth kinetics. A total of 8 schott flasks varying inoculum concentrations were maintained at 28 ° C and 100 rpm. Samples were taken every 24 h and viability measured by Trypan Blue exclusion method. **Results and Discussion:** The adaptation process was made without major difficulties. Only in two passages (with 65 % and 96 % of IPL 41), it was necessary to wait an additional week to attain the expected adaptation. S2MtRVGP-His cells adapted to 100% IPL41 were frozen in a work bank. Growth kinetics performed on SF9000II showed growth rate (μ_x)of $0.029 \pm 0.006\ h^{-1}$, and $0.016 \pm 0.004\ h^{-1}$ for cultures inoculated with 10^6 cells / mL or 2×10^6 cells / mL, respectively. On IPL41 μ_x were $0.032\ h^{-1}$, and $0.028\ h^{-1}$, respectively. Morphological changes in cells were noticed during adaptation process: Among a large majority of cells almost perfectly spherical, it were noted some elongated cells. From growth kinetics results, higher values of μ_x showed that it is better to work in both culture media with an inoculum of 10^6 cells / mL, providing higher growth rates and longer exponential phase of growth. Further studies with recombinant expression induction will be performed and RVGP productivity analysed.

Supported by FAPESP



6.26 The toxin VapC of the toxin-antitoxin system VapBC from*Leptospira interrogans* is a ribonuclease that cleaves specifically the initiator transfer RNA

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Introduction: Toxin-antitoxin (TA) *loci* are ubiquitous in prokaryotes. They are bicistronic operons that encode a stable toxin and an unstable antitoxin. TA function remains controversial but it has been accepted as a novel mechanism of bacterial growth modulation in which the action of TA *loci* enable the entrance in a reversible bacteriostatic state induced by stressful conditions. The *vapBC* operons constitute the largest family of bacterial TA modules, grouped due to the homology by the PIN domain of the toxin, which is thought to act as ribonuclease. Toxin activity toward RNA affects translation causing growth inhibition but specific targets are mostly unknown. Due to its toxic activity over bacteria the expression of VapC without the cognate inhibitor VapB is considered unfeasible. We have cloned the *vapBC locus* of *L. interrogans* and expressed the cognate proteins in order to characterize them. **Objectives:** Produce the toxin VapC in a soluble and correct folded form. Verify whether the VapB neutralization of the inhibition of cellular growth caused by VapC is related to the binding between the two molecules. Find out leptospiral VapC target and characterize its activity. **Methods:***vapC*, *vapB* and the*locusvapBC* of *L. interrogans*were amplified, cloned in pAE vector and expressed in *E. coli* BL21(DE3). Proteins were purified by Ni^{+2}-Sepharose chromatography after previous refolding by pressurization in the case of VapC. Structural integrity was analyzed by circular dichroism spectroscopy (CD). Interaction between VapB and VapC was tested by pull-downassay and affinity blotting. Activity of VapC was tested against *E. coli* rRNA and $tRNA^{fMet}$. **Results and Discussion:** VapB was obtained in soluble form while VapC was expressed insoluble. Refolding of VapC was achieved by pressurization in presence of L-arginine. CD showed a mixture of α-helix, β-strand and coil in VapB secondary structure and a predominance of α-helices in VapC, suggesting that they are likely folded. Coexpression of VapB and VapC confirms that the toxic effect of VapC on *E. coli* growth is neutralized by VapB. This neutralization was probably caused by their interaction as demonstrated by a pull-down assay. The binding between recombinant VapB and VapC was observed *in vitro* by affinity blotting indicating that the proteins kept their natural binding affinity. VapC did not present ribonuclease activity toward *E. coli* rRNA but cleaves $tRNA^{fMet}$ and therefore probably affects bacterial growth rate via inhibition of translation. VapC activity depends on the presence of Mg^{+2} and is inhibited by VapB.

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6.27 Comparative study of mycobacterial promoters using GFP as a reporter of gene expression for the development of recombinant BCG vaccines

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Introduction: Tuberculosis (TB) is an infectious disease that affects the entire world. The only prevention of this disease is through vaccination with BCG, the only vaccine used today. However, BCG has limitations, such as the decline in protective immunity over time, leading to research for the development and improvement of vaccines against TB. With the advances in molecular biology, researchers are investing in recombinant BCG vaccines for providing greater protection than BCG against tuberculosis. Expression systems are important issues for recombinant BCG constructs, being the promoter an important element determining level of antigen production and the induction of immune responses. **Objectives:** To compare the activity of various promoters, including a new mycobacteriophage promoter pL5, using the green fluorescent protein (GFP) as reporter of gene expression. **Methods:** The *gfp* gene and the promoters *pAN*, p*αAg*, p*hsp* and *pL5* were amplified by PCR and the fragments subcloned as expression cassettes into pGEM-T-easy (pGEM) before being inserted into the mycobacterium vector pLA71 (pNN71) and selected by transforming *E. coli* DH5α. The plasmids were extracted for restriction analysis and sequencing to confirm the positive clones and then used to transform both *M. smegmatis* and *M. bovis* BCG followed by fluorescence analysis using microscopy and flow cytometry. **Results and Discussion:** It was possible to clone the cassettes *pAN-gfp*, *pBlaf-gfp*, p*αAg-gfp*, p*hsp-gfp* and p*L5-gfp* (pΣ) in the vector pLA71 (pNN71-pΣ). The pNN71-p*AN-gfp* and pNN71-p*Blaf-gfp* had previously been cloned in the lab. Despite these promoters being specific to mycobacteria, interestingly, we have also observed some GFP activity even in *E. coli* transformed with pGEM or pNN71 plasmids and such activity was dependent on each promoter. These promoters in the mycobacterium vector pNNL71 have shown different features after transfection into *M. smegmatis* or BCG. It was possible to observe *M. smegmatis* fluorescence for almost all constructions, except *pαAg*-gfp. In the case of BCG, it was not possible to select BCG clones using pNN71- p*L5-gfp*. In general, the different promoters have shown their strength in a gradual scale from weak, such as *pAN* to intermediate, such as *phsp60,* to higher expression levels, such *pL5*. The results obtained so far with the various constructs are promising, as they open the prospect of using these plasmids to test various heterologous antigens based on the same backbone vector, therefore, allowing easier characterization of their expression. In addition, the plasmid pNN71-*pL5-gfp* showed potential to be used in both expressions in *E. coli* and in *Mycobacterium* systems.

Supported by BNDES, CNPq, Fundação Butantan

6.28 Evaluation of immunoprotective activity of rLIC12880 and rLIC12238 proteins in the hamster model of leptospirosis

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Introduction: *Leptospira interrogans* is the etiological agent of leptospirosis, a zoonotic disease of human and veterinary concern. In the urban settings, rodents are the most important carriers of the disease because they continuously shed live leptospires in their urine. Humans can be infected through contact with soil or water contaminated with urine containing live leptospires. Since the control of the rodents and sanitation measures are not easily implemented, the development of reliable vaccine is necessary to combat the leptospirosis. **Objectives:** The aim of this project is to evaluate the immune response promoted by two recombinant proteins (rLIC12880 and rLIC12238) in hamsters. **Methods:** Twelve Golden Syrian hamsters (6 - 8 weeks old) were immunized subcutaneously with 50 µg of recombinant protein adsorbed in 10 % Alhydrogel (2% $Al(OH)_3$), used as adjuvant. One booster injection was given after two-weeks with the same preparation of recombinant protein. Negative-control group of hamsters were injected with PBS in 10% Alhydrogel. As a positive control, a group of hamsters was immunized with killed whole-leptospires (bacterin-like vaccine). Before the challenge, the hamsters were bled from the retro-orbital plexus and the pooled sera were analyzed by enzyme-linked immunosorbent assay (ELISA) for determination of antibody titers. Two weeks after the second immunization, the hamsters were challenged with an intraperitoneal inoculum of 2.5 x 10^3virulent leptospires (*L. interrogans* serovar Kennewicki strain Pomona Fromm). After challenge, the animals were monitored daily for clinical symptoms until 28 days post-infection. **Results and Discussion:** Evaluation of recombinant proteins protection in hamster model followed by challenge with virulent leptospires was performed in two independent assays. In the first experiment, 33, 50 and 8% of the animals immunized with rLIC12880, rLIC12238 and PBS (control-group) survived, respectively. In the second experiment, 16.5 and 25% of the hamsters immunized with rLIC12238 and PBS (control-group) survived, while no survival animal was seen with rLIC12880 protein. In both experiments, heat inactivated whole-cell leptospires (bacterin) afforded 100% protection. The evaluation of the kinetics of the survival curves by Kaplan-Meyer showed that the protection conferred by rLIC12880 and rLIC12238 was not statistically significant in any experiment.

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6.29 Improvement of polysaccharide production by *Haemophilus influenzae* type b in a chemically defined medium

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Introduction:*Haemophilus influenzae* type b (Hib), a Gram-negative bacterium, is responsible for significant morbidity and mortality in young children and elderly. The capsule, a polysaccharide consisting of repeating units of ribosyl ribitol phosphate (PRP), is the major factor of virulence. Hib vaccine is based on PRP linked covalently to a carrier protein, which makes this product expensive, demanding high productivity in the PRP production. The current culture medium used by the pharmaceutical industries to produce Hib PRP is based on soybean peptone and yeast extract. Previous studies of *H. influenzae* using chemically defined medium were dedicated to genetic and metabolic studies, however neither related to industrial PRP production by Hib. **Objectives:** to establish chemically defined medium for growth of Hib and PRP production to use in the vaccine formulation. **Methods:** The experiments were carried out based on previous works used for genetic transformation (M_{ic}) and a RPMI medium-base tissue culture media adapted for *H. influenzae* development. Assays were divided in four different groups: (1) Control - M_{ic} medium, (2) Medium 1 supplemented with: Glutamine (Gln), Isoleucine (Ile), Phenylalanine (Phe), Tryptophan (Trp), Valine (Val) and Cysteine (Cys) individually and in a pool of all of them, (3) Medium 2 supplemented with vitamins: Biotin, Colin, Folic acid, Nicotinamide, p-aminobenzoic acid and Riboflavin. The inoculum was activated by static incubation at 37°C with low oxygen tension for 10h and incubated overnight at 37°C, 250 RPM. An amount of this inoculum was transferred to flasks containing 100 mL of each medium in order to reach OD_{540nm} of 0.1, and growth was followedat 37ºC, 300 RPM by reading the OD_{540nm} every hour. Production of PRP was measured by modified Bial´s method. **Results and Discussion:** In the control medium (1) thegrowth of Hib showed non exponential profile indicating limitation of some nutrient achieving an OD_{540nm} of 4.3 and producing 182.38 mg/L of PRP. The addition of all amino acids mentioned (2) promoted a growth profile with characteristic microbial growth phases, proving the requirement of more nitrogen sources in the medium, achieving OD_{540nm} of 3.7 and PRP production increasing to 223.5 mg/L, which is 50% of PRP produced in complex medium MMP. Individually, the absence of Cys and Phe showed no significant effect in the growth and PRP production. Addition of vitamins (3) showed no difference in the parameters studied with PRP production around 200 mg/L. Supplementation of M_{ic} medium with Glu, Ile, Trp and Val promoted better growth than the control medium and is a strong candidate to improve PRP production .

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6.30 Adaptation to Serum-Free Culture of HEK 293T and Huh7.0 cells.

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Introduction: The fetal bovine serum (FBS) is a component of higher value added to the medium, which may cause difficulty in the process of recovery and purification of bioproduct. The adaptation of cellular lineages to culture FBS or animal protein free medium can allow optimization of cell growth and expression of heterologous genes, facilitating recovery of recombinant proteins expressed in these cells. **Objectives:** To adapt HEK293T and Huh 7.0 cells in serum free media. To measure parameters, like cellular growth, viability and glucose, glutamine and lactate production/consumption, in these adapted cells. **Methods:** Adaptation of HEK 293T and Huh 7.0 cells was done in four SFM, Pro293TMa, VP-SFM, SFM-Hybridoma, and CHO-S-SFM II. Kinetics parameters of adherent cells were analyzed in duplicate for 5 days in 6 well plates with 2 mL of medium. The initial cell concentration in all experiments was 2×10^5 cells/mL **Results and Discussion:** We established protocols to adapt HEK293T and Huh7 to growing cells in SFM. By changing gradually of medium, we obtained two lines adapted HEK 293T to two SFM (SFM-Hybridoma, CHO-S-SFM II) and 4 Huh 7.0 line to four SFM. The Huh 7.0 cells showed morphology and growth better than HEK293T cells. The cells was evaluated and the maximum growth of the line Huh-7.0 in the SFM-Hybridoma and CHO-S-SFM II were 120hrs with 1.0×10^6 cells / ml with viability higher than 90%. The maximum concentration HEK 293T cells and viability were similar to Huh 7.0 cells, but those achieved maximum peak of growth in different times. The SFM CHO-S-SFM II obtained maximum growth in 96 hours and Hybridoma-SFM in 72hrs.

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6.31 Tracking the factor XI in fraction of plasma protein purification process for IgG obtainment

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Introduction: A plant for plasma fractionation is in construction at the Instituto Butantan with financial support from the State of São Paulo Government. For the new factory, a modern production process was drawn, using primarily chromatography for obtaining the biopharmaceuticals IgG, albumin and the coagulation factors VIII and IX. The process is innovative and differentiated from the processes commonly used in hemoderivatives industrial plants, which are based in modifications of the Cohn methodology, fractionation by precipitation in cold temperatures and various ethanol concentrations. A pilot plant was assembled to establish the IgG process. Thromboembolic events were recently described after IgG treatments and the pro-coagulant activity was associated with contamination of IgG preparation with coagulation Factor XI (FXI). **Objectives:** Our research proposes the verification of the presence of FXI in the IgG preparations obtained through the chromatographic process previewed to be used in the industrial plant. **Methods:** The complete or partial process for IgG obtainment was performed in the pilot plant. Also the first steps of the protein purification are being reproduced in bench scale, using the chromatographer Akta purifier for the control and register of the process. The presence of FXI in samples of IgG from pilot scale and samples from different fractions of the chromatographies were investigated using SDS PAGE and western blot. The FXI activity was measured through activated partial thromboplastin time (aptt) and chromogenic method. **Results and Discussion:** There was no indication of FXI in the samples of IgG obtained in pilot scale. Some samples of the chromatographies performed at bench scale were shown to be instable post-freezing, presenting protein precipitation and complex formations with jelly appearance. Immediately after the first chromatographic step the second will be carried out and the FXI activity tests also immediately done. The use of heparin as anticoagulant in plasma interferes in the FXI dosages, mainly in samples from the first chromatography. In the second step, the interference of heparin was diminished, and there was indication of the presence of FXI in the flow through and second fraction of the chromatography. Heparin is necessary for the industrial process, but in order to trace the presence of FXI in the chromatographic fractions, the process is being performed in absence of Heparin.

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6.32 An innovative process to purify polysaccharide from *Haemophilus influenza*e type b through tangential flow filtration technology

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Introduction: *Haemophilus influenzae* b (Hib) is an encapsulated Gram negative, pathogenic bacterium responsible for pneumonia and meningitis in infants and elderly worldwide. The capsular polysaccharide (PRP) is its main virulence factor; on the other hand it is used as the antigen in vaccine formulation after chemical conjugation to a protein. The classical PRP purification process includes several fractionation steps with ethanol, phenolfor removal of proteins (Prt) and nucleic acids (NA), detergent for removal of lipopolysaccharides and the use of centrifugation/ultracentrifugation, which result a very onerous and laborious process. Improvements in the PRP purification process have been developed by our research group. For instance, the reduction of ethanol fractionation to two (30% and 80%), and more recently the substitution of centrifugation by hollow fiber tangential flow microfiltration (TFMF). In both cases were employed anionic detergent (deoxycholate) and enzymatic digestion. According to World Health Organization (WHO) the main requirement for purified PRP is a relative purity of over 100 related to proteins (RP_{Prt}) and nucleic acids (RP_{NA}), where RP means the amount of PSb per amount of Prt or NA. The recovery of PRP was calculated as the percentage of the final recovered PRP in relation to the initial step. **Objectives:** To optimize the polysaccharide purification process by using ethanol precipitation in the presence of strong detergent (SDS) and removal of precipitates by TFMF. **Methods:** Cell-free supernatant from bacterial culture (6L) was concentrated/diafiltrated by tangential flow ultrafiltration (TFUF) on a 100 kDa cut-off membrane, followed by 30% (v/v) ethanol precipitation in presence of 0.5% SDS, and removal of insolubles by TFMF using hollow fiber of 0.2 µm pore. The microfiltrated fraction containing PRP was concentrated by TFUF at a 50 kDa cut-off and submitted again to precipitation of PRP at 80 % (v/v) of ethanol. The PRP was solubilized with pure water and recovered through the same TFMF. The microfiltrated water soluble PRP was concentrated again by TFUF resulting in the purified PRP. **Results and Discussion:** The purified PRP presented a RP_{Prt} of 321 and a RP_{NA} of 405, attending the WHO specification, and a final recovery of 35.1%. The introduction of SDS, a strong detergent, facilitated the action of ethanol and the removal of impurities by TFMF. This innovative method makes it possible to eliminate the enzymatic digestion step, yielding a cost-effective and more practicable process for scaling up.

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6.33 qRT-PCR for titration of replication-defective recombinant Semiliki Forest Virus

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Introduction: The finding that the SFV was able to infect many cell types with high efficiency in gene delivery and that the use of a strong viral promoter allowed the amplification of heterologous RNA into the host cell led to the development of the SFV expression vector. To attain reproducible results it is essential to determine the titer of SFV particles before using for infection. SFV titration is not an easy procedure as particles are defective and do not replicate or form plaques. The qRT-PCR technique can be a good alternative for the titration of SFV particles. **Objectives:** To standardize a method for qRT-PCR titration of non-replicative SFV particles, directed to the conserved region of the gene coding for the nonstructural protein 1 (nsp1). **Methods:** The standard curve for SFV-RNA quantification was obtained by six dilutions of 2 µg of pSFV-RVGP plasmid, generating the standard dilutions containing 6 x 10^7to6 x 10^2copies at each 3 µL. Four independent runs with three replicates of each standard dilution were performed on real-time fluorescence detector termocycler. Reaction was set-up with Power SybrGreen kit(Life Technologies), 3 µL cDNA sample, 50 nMforward primer SFV-S2, 50 nM reverse primer SFV-R2 in 15 µL total reaction volume. qPCRwas performed at 95 ºC / 10 minutes, 34 x (95 ºC /15 s, 55 ºC /15 s, 60 ºC /15 s), and the fluorescence measured at 60 ºC. After amplification, melting curve (60 ºC a 95 ºC) was developed in order to verify reaction specificity, with the amplified fragment (145 bp) presenting dissociation at 83 ºC ± 0.3 ºC. **Results and Discussion:** The standard curve showed the basic requirements necessary for qRT-PCR, namely, the use of at least five dilutions of standard and a slope of standard curve preferably between -3.3 and -3.8 and R^2 value of 0.99. The reproducibility was 0.23 ± 0.06 Ct inter-assays and 0.10 ± 0.06 Cts intra-assays. Different SFV vectors were efficiently quantitated by the method, showing that it is applicable to all SFV constructs. These data showed that the fast and reliable qRT-PCR method can be used for SFV titration.

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6.34 Evaluation of the expression of RVGP in different mammalian cells using the Semliki Forest virus (SFV)

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Introduction: The Semliki Forest Virus (SFV), the genus *Alphavirus* is widely used as a vector expression of heterologous proteins in mammalian cells. The rabies virus glycoprotein (RVGP), recognized as an antigen capable of conferring immune response against rabies, was chosen as a target gene in this approach. **Objectives:** To evaluate the expression of RVGP in different cell lines using the SFV system (SFV-RVGP); to determine the best conditions for the expression of the heterologous protein in the cell culture and viral infection. **Methods:**Two different plasmids were used: an expression plasmid containing SFV genes coding for nonstructural proteins and the RVGP gene, and a helper plasmid containing SFV genes coding for structural proteins. *In vitro* transcription was performed and RNAs were co-transfected in BHK-21 cells, for generation of SFV-RVGP. They were then activated and used to infect BHK-21, Huh 7.0, Vero and L929 cells, and to induce the heterologous protein expression (RVGP). Measures of RVGP were done by ELISA, 24 and 48 h after infection. **Results and Discussion:** Using the SFV-RVGP method of expression, we evaluated the RVGP production in different cells (BHK-21; Huh-7; L929; Vero), using different MOIs (1, 10, 15 e 50). The experiments were performed in duplicate in 6 wells plate in CO2 incubator at 37 ° C. The cell inoculum was of 7x1E5 cells/well with a working volume of 2 mL. Based on these results, BHK-21 cells showed the best rate expression RVGP, and the MOI 1 proved better than the others. A similar phenomenon occurred with Vero cells, but with lower production. However, in the process of infection of Huh-7 cells, and L929 cells, the expression of RVGP increased as the MOI was higher, being greater when we infected them with MOI 50. Another relevant fact is that the Huh-7 cells showed higher expression rate 48 h after infection, unlike the other tested strains obtained the best indices after 24h.

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6.35 Antischistosomal bioactivity prospection in rhodophyceae and phaeophyceae seaweeds extracts

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Introduction: Schistosomiasis still remains a serious problem in developing countries, as a debilitating neglected tropical disease that afflicts over 200 million people worldwide. **Objectives:** As the treatment for this disease is sustained mainly by only one drug, praziquantel, we aimed to achieve possible alternative drugs, so there were tested Rhodophyceae(red) and Phaeophyceae (brown) algae extracts against *Schistosoma mansoni.* **Methods:** Algae extracts of five different species, identified as *Plocamium brasiliense*, *Chondria littoralis* and *Spyridia hypnoides* (red algae) and *Dictyota menstrualis* and *Dictyota dichotoma* (brown algae), were obtained employing supercritical CO_2 extraction techniques. In order to evaluate schistosomicidal potential of extracts, adult male *S. mansoni* worms were recovered by perfusion from hamsters 45 days after cercariae infection. The worms were placed individually in 24-well culture plates where they were exposed to 0.5 mg/mL of extracts in RPMI1640 medium. Parasites were kept for 120 hours and were monitored 2 hours after exposure ion and every 24 h to evaluate parasite's death. **Results and Discussion:** The extracts of *D. menstrualis* and *P. brasiliense* leaded to death all the worms after only two hours of exposure, while *D. dichotoma* took 24h to reach this effect level. In comparison, the extract of *C. littoralis* only reached total mortality after 96 hours of exposure and *S. hypnoides* extracttook 120 hours of exposure to achieve the same effect.As all the algae extract showed a remarkable effect against *S. mansoni*, mainly *Plocamium brasiliense* and both species of *Dictyota*, the next step is to test the active extracts, against parasite couples, in lower concentrations and bioguided fractionation in order to identify a pharmacological effective molecule presenting antischistosomal activity.

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6.36 Development of rapid immunochromatographic test, using "*dipsticks*" for diagnosis of *Streptococcus pneumoniae*

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Introduction: The *Streptococcus pneumoniae* is the most common bacterial respiratory pathogen spread worldwide and is the major cause of infant mortality, with more than 1.2 million deaths in children under the age of 5 years old, mainly in developing countries. The World Health Organization recognizes the development of rapid diagnostics as an essential strategy in the optimization process of public health systems and the control of epidemics. In this context, rapid immunochromatographic assays or lateral flow test (dipstick) based on capillary action and colored particles, involving relatively simple technology for antigen-antibody reaction in a single solid phase, are alternatives to traditional methods for identification of pathogens and antigenic molecules. **Objectives:** The purpose of this study is the development of a dipstick test for diagnostic of *S. pneumoniae*. **Methods**: The dipstick was developed using mice monoclonal (MAbs) or polyclonal antibodies (PAb) against specific antigens of *S. pneumoniae*. Colored colloid microspheres (Estapor® Merck Millipore) were conjugated, by simple adsorption, to (i) Mabs against streptococcal pneumolysin (Ply); (ii) polyclonal serum anti-Ply or (iii) polyclonal serum against a whole cell pneumococcal vaccine in development in our laboratory (anti-WCPV); to be used as detector antibody, immobilized in a glass fiber located in one of the extremities of the dipstick. These conjugated antibodies binds to the antigen samples (supernatants of cultures or bacterial suspensions of *S. pneumoniae*) to form a complex which is captured by the capture antibody (Mab anti-Ply; Pab anti-Ply or anti-WCPV) immobilized in a solid phase, a nitrocellulose membrane (MN). The samples are allowed to flow through chromatography in the NM, the conjugated antibodies bind to the antigens, that and can be catched by the MAb immobilized in the test line. Anti-mouse IgG was immobilized in the nitrocellulose membrane, as control line.The presence of two bands in the NM was interpreted as a positive result and a single band (control line) was considered as negative. **Results and Discussion:** The dipstick assembled with conjugated anti-WCPV and Mab anti-Ply as capture presented a detection limit of 10^4 cells/ml of pneumococcus, while the system anti-Ply as conjugated/ Mab anti-Ply as capture was able to detect at least 9.7ng/ml of Ply in culture supernatant of *S. pneumoniae.* The high sensitivity and the rapidness of the test detection (within 20 min) suggest that it might be a useful platform for further development of point-of-care and diagnostic applications.

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6.37 Expression Analysis of Three Novel Proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is an important zoonotic disease caused by infection with pathogenic *Leptospira* spp. bacteria. Several outer membrane proteins have been identified among the sequences of *L. interrogans* serovar Copenhageni and these proteins might be involved in host-bacteria interactions.**Objectives:**These studies aim to evaluate the expression of three genes encoding for hypothetical proteins (LIC11360, LIC11009 and LIC11975) together with protein localization in bacteria. **Methods:** The genes were cloned and expressed in *Escherichia coli* strain BL21 (SI)using the expression vector pAE. The recombinant proteins were purified using affinity chromatography. The secondary structure content of the purified proteins was evaluated by circular dichroism spectroscopy; the reactivity of recombinant antigens with human leptospirosis serum samples was analyzed by ELISA and the cellular localization was performed by proteinase K accessibility assay. Evaluation of transcripts and protein expression was assessed in several leptospiral strains by RT-qPCR and Western blotting, respectively. **Results and Discussion:** RT-qPCR analyses showed that the three genes are expressed mainly in *L. interrogans* strains. The native protein LIC11009 was detected only in the bacterial extracts of *L. interrogans* by Western blotting. Purified recombinant proteins rLIC11009 and rLIC11360 exhibited predominantly β – strand structures while rLIC11975 was mainly alpha helix. The three proteins were susceptible to protease treatment suggesting that these proteins are surface exposed. They were recognized by IgG antibodies present in human leptospirosis serum samples, at convalescent phase, suggesting that they are expressed during infection. These proteins might have a role in leptospiral pathogenesis.

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6.38 HER2 Gene Isolation and Cloning Targeting Gene Silencing by *RNAi* as an Alternative Therapy

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Introduction: The advances in biotechnology, especially the possibility of manipulation and interspecies gene transfer, are shown as an important tool to overcome the conventional therapeutic methods with the development of new drugs and therapies, to the development of more sensitive diagnostic and the depth study of various diseases. The breast cancer is the malignancy with the highest incidence and the second cause of death in female individuals worldwide and gain of function of the *HER2* gene (*v- erb - b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog)* is one of the most significant genetic changes to promote malignant breast tumors to confer more aggressive biological character, poorer prognosis, greater likelihood of recurrence and lower survival. Treatment for the disease has evolved considerably in recent years, however, the emergence of therapeutic interventions more efficient, less invasive, and less costly is needed. In this context, biotechnology combined with gene therapy may emerge as an alternative to existing treatments. **Objectives**: biomolecular assays for isolation and cloning of the *HER2* gene from peripheral blood samples of patients with breast cancer promoting future prospects for the realization of other tests of genetic manipulation in expression vectors. Therefore, addressing the main characteristics of biotechnology and its tool interference by *RNAi* (RNA interference), raising a discussion about their current use and highlighting recent advances and studies in the field of gene therapy for neoplastic diseases. **Methods**: This is a prospective study in which DNA and RNA were extracted from total peripheral blood samples with specific commercial kits, amplification of the gene fragment study by Polymerase Chain Reaction, introduction of the isolated fragment into a plasmid vector and transformation in competent bacteria *E. coli* by electroporation, clones were selected by IPTG and X -gal enzymatic digestion occurred and the restriction enzyme EcoR-I. **Results and Discussion**: Isolation of gene fragments from total DNA was successful, as well as the subsequent steps, so obtained clones containing the fragments of interest considering the great contribution to the health area. Furthermore, cloning of the fragments isolated from total RNA, which was a limitation of this study, would favor the development of alternative therapies based on gene therapy.

6.39 Kinetic study of BHK-21 cells adapted to Serum Free Media

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Introduction: Supplementation of culture media with fetal bovine serum (FBS) has been necessary for cell growth in vitro. However, FBS, used more often, presents some disadvantages, such as the potential to induce hypersensitivity, the variability of serum batches and the risk for contamination, besides the high cost of a good quality serum. For these reasons, current biotechnological approaches of cell culture need to avoid the use of serum. **Objectives:** Our aim was to establish and to study mammalian cell lines adapted to serum free medium for the expression of recombinant proteins. Thus, we assessed cell growth, nutrient consumption and metabolite production kinetics in the cell culture media tested. **Methods:** In this work, BHK-21 (adherent) and BHK-21 C13 (suspension) cells were adapted to different serum free media (SFM) using sequential adaptation approach. BHK-21 (adherent) cells were adapted to 5 different SFM: VP®-SFM, Hybridoma®-SFM, MAb® medium and CHO®-S-SFM II; while BHK-21 C13 (suspension) cells were adapted to 2 SFM: Hybridoma®-SFM and Pro293a®. Kinetics parameters of adherent cells were analyzed in duplicate for 5 days in 6 well plates with 2 mL of medium. Suspension cells were analyzed in 25 cm2 T-flasks with 7 mL of medium and spinner flasks with 50 mL of medium. The initial cell concentration in all experiments was 2×10^5 cells/mL. **Results and Discussion:** Maximum cell concentrations of adherent cell cultures were reached after 96 hours of cultivation: 2×10^6 cells/mL; 2.83×10^6 cells/mL; 2.37×10^6 cells/mL; 1.36×10^6 cells/mL; 2.49×10^6 cells/mL for Hybridoma, MAb, CHO-SFM II, VP-SFM and Control (DMEM with 10% of FBS), respectively. For suspension cells in T-flasks, the maximum cell concentrations reached after 96 hours were 1.76×10^6 cells/mL; 1.18×10^6 cells/mL and 1.77×10^6 cells/mL for Hybridoma, Pro293a and Control (DMEM+IMDM+5%FBS) respectively. Whereas for suspension cells in spinner flasks the cell concentration achieved were 5.10×10^6 cells/mL; 2.20×10^6 cells/mL; 4.78×10^6 cells/mL, respectively. Metabolic analysis showed differences in the consumption of nutrients and production of metabolites among adherent and suspension lineages.The cell concentrations achieved in all SFM were similar to those obtained in the media containing serum, suggesting a good adaptation of BHK-21 to the serum free media.

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6.40 Identification a OmpA-like protein with potential role in leptospiral pathogenesis

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Introduction: Leptospirosis is a worldwide zoonosis regarded as a major public health problem. Measures to control the disease are difficult to implement. The development of new strategies to prevent and control the spread of disease is urgently needed. Accordingly, prophylactic vaccines or immunotherapeutic emerge as strong candidates to solve the problem. For this reason, currently research has focused to identify conserved antigens that are involved in host-pathogen interactions. **Objectives**: This project aims to assess the functional properties of the coding sequence LIC13479 of *L.interrogans* serovar Copenhageni, identified by bioinformatics as a putative outer membrane protein. **Methods:** The LIC13479 sequence was amplified by PCR using specific primers, cloned into the expression vector pAE and used to transform *E.coli* DH5-α. Plasmids containing cloned DNA were introduced in *E. coli* strains for protein expression studies. After purification of the recombinant protein, mice were immunized and antibodies anti-LIC13479 evaluated. **Results and Discussion:** The coding sequence LIC13479 was successfully cloned, without the sequences corresponding to the signal peptide. The expected recombinant protein was detected by 12% SDS-PAGE and expressed in the insoluble form. Protein purification was successful and produced 1.5mg/L of recombinant protein per bacterial culture. Analyses show that the recombinant protein LIC13479 is capable to stimulate antibody immune response in Balb/C mice and, in addition, is recognized by infected human and hamster sera. Our data indicate that this protein is expressed during leptospiral infection.

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6.41 Pluripotent stem cell niches virtually exist at early post-implanted stages in the central neuronal system of mice

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Introduction: It is of a common knowledge that pluripotent cells can be isolated of inner cells mass (ICM) of blastocyst and/or epiblast of pre- implanted embryo. A few stem cells, which retain pluripotent potential, were also found in adult tissues of bone marrow, dental pulp and brain. Multipotent stem cell niches in neural system of mice, more precisely, were found in subventricular zone (SVZ) composed by neural stem cells, intermediate progenitors, neuroblasts, astroglial and ependyma cells. **Objectives:** We aimed at identification of stem cell niches in the central neural system (CNS) at early post-implanted stages of mice and isolation of stem cells from CNS similar those isolated in ICM. **Methods:** Three (early and late) fetal developmental stages of mice C57BL/6 were used 12 d.p.c., 15 d.p.c. and 18 d.p.c. Using the tissues from mice CNS the expression of such proteins as Oct4, Nanog and Sox2 and respective genes were performed by both immunohistochemistry and PCR. Next, the stem cell cultures from CNS tissues of mice fetus were isolated under maintained under conditions appropriate for ICM cells cultured *in vitro*.**Results and Discussion:**Both analyses coincidence the expression of all studied markers in brain vesicles (prosencephalon, mesencephalon, robencephalon) at 12 d.p.c. The Oct3/4 and Sox2 positive few cells were also observed in brain vesicles (prosencephalon, mesencephalon, robencephalon) at 15 d.p.c. No expression of pluripotent stem cell markers were found at 18 d.p.c. The cells, which showed embryonic-like morphology were immunopositive to Oct-4 and Nanog, and were isolated from fetal tissue at12d.p.c, only. Our results suggest that in mice fetal CNS, the pluripotent cells can be found in reminiscent embryonic-like stem cells niches (e.g. ICM). We also showed that it is possible to isolate and cultivate these cells *in vitro*. Our finding implicates that stem cell niches can virtually exist in early pos-implanted stages and they probably are regulated by the same pluripotent genes.

Supported by Fapesp

7. Cellular Biology and Genetics

7.01 Mastoparan induces apoptosis in melanoma B16F10-Nex2 cells via intrinsic mitochondrial pathway and displays antitumor activity *in vivo*

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Introduction: Mastoparan (INLKALAALAKKIL) is aα-helical and amphipathic tetradecapeptide obtained from the venom of the wasp *Vespula lewissi*. These peptides exhibit a wide variety of biological effects, including antimicrobial activity, histamine release from mast cells and induction of a potent mitochondrial permeability transition. **Objectives:**The aim of this study was to evaluate the cytotoxicity in B16F10-Nex2melanoma cellsand *in vivo* antitumor effects of mastoparan. **Methods:**Cell viability was evaluated by MTT assay on B16F10 cells with or without the pre-treatment with the antioxidant N-acetylcysteine (NAC). Tumor cell death by apoptosis was determined using the Annexin V-FITC/PI assay.The loss of mitochondrial membrane potential ($\Delta\Psi m$) was evaluated by staining cells with tetramethylrhodamine ethyl ester (TMRE) and quantified by flow cytometry. Generation of reactive oxygen species (ROS) was performed usingoxidative fluorescent dye (DHE) and fluorescence microscopy (FM). DNA fragmentation was evaluated by electrophoresis in an agarose gel, and chromatin condensation was assessed by DAPI staining.We have used Western blotting to evaluate mastoparan induced cell death signaling. Finally, we utilized a tumor grafted murine melanoma syngeneic model to investigate the antitumor activity of mastoparan *in vivo*. **Results and Discussion:**We showed that B16F10 cells treated with mastoparan was cytotoxic with an $IC_{50}$of 165 μM. In addition, 165 μM of mastoparan was able to induce increase of 44.8 ± 4.5% positive cells populations for annexin V. Furthermore,165 μM of mastoparan induced a 49.7% decrease of $\Delta\Psi m$. Mastoparan, also displayed a 74.76% increase in the generation (ROS) and attenuated cytotoxicity (IC_{50}295 μM).In addition,165 μM of mastoparan induced DNA fragmentation and simultaneously chromatin condensation in 65.87% cells. Also, mastoparan increases the expression of cleaved caspases-3, -9 and -12, PARP, Bim, Bak, Cytochrome c as well as decrease expression phospho Bad (S112), VDAC, PHB1 and Bcl-2. Most importantly, mastoparan (5mg/kg) reduced the growth of subcutaneous melanoma *in vivo* (70.29%) and increased mice survival in 28.26%. Statistical analysis was performed using Student's t test with Prism 5 software. Thus, the results indicate that mastoparan induces apoptosis in melanoma cells through intrinsic mitochondrial pathway and also protected mice against tumor development.

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7.02*Cis*-acting genetic elements at or near the *Pas1* locus control *Kras2* mutations and gene expression in lung tumors from mice selected for acute inflammatory response.

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Introduction: AIRmax (high response) and AIRmin (low response) mice, genetically selected for the intensity of acute inflammatory response (AIR), show divergent susceptibility for chemically-induced tumors, suggesting that genes which control acute inflammatory reactivity may modulate predisposition to tumorigenesis. AIRmax and AIRmin carry the resistance and susceptibility haplotypes, respectively, at *Pas1* (*Pulmonary adenoma susceptibility1*), the major locus regulating susceptibility to lung tumorigenesis in mice. The oncogene *Kras2* maps at *Pas1,* and mutations of this gene are associated to lung tumor development. **Objectives:** Identify, through linkage analysis, chromosomal regions which control the mutability of *Kras2* and the expression levels of *Kras4A* and *Kras4B* isoforms in lung tumors of intercrossed F2 (AIRmax x AIRmin) mice. **Methods:** Mice were treated at 7 days of age with 300 mg/kg urethane and after 9 months lung tumors were excised for DNA (n=500) and RNA (n=110) extraction. *Kras2* activating mutations at codons 12, 13 and 61 were identified by DNA pyrosequencing. Expression levels of *Kras* isoforms and *Lyrm5* (also mapping inside *Pas1*) were determined by qPCR. A panel of 1449 SNPs (single nucleotide polymorphisms) distributed in the whole genome, was tested for association with the mutability and expression phenotypes. **Results and Discussion:** One region at chromosome 6 (near to *Pas1*) was associated with *Kras* mutations (LOD 4.67). The expression levels of *Kras4A*, *Kras4B* isoforms and *Lyrm5* showed association with *Pas1* in chromosome 6 (LOD 3.16, 2.36 and 8.43 respectively). Results suggest the participation of *cis*-acting elements at or near *Pas1* in the regulation of *Kras2* mutability and expression.

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7.03Reprogramming of murine melanoma cells by Yamanakas transcription factors into embryonic state

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Introduction: Currently many studies support the idea that embryonic and cancerstem cells shared such characteristics as plasticity and undifferentiated phenotype, which are regulated, at least partially, by common mechanisms. These similarities between cancer and pluripotent cells are corroborated with cancer stem cell theory. Several reports have demonstrated the reversion of the metastatic phenotype of human melanoma cells by the embryonic microenvironment. The use of reprogramming is a good starting point, which can contribute significantly into understanding the main molecular mechanism underlying the pathogenesis of this aggressive tumor and to develop novel biological based strategies for anticancer therapies. **Objectives:**In present work, for the first time, we used non-viral vector, which contain four Yamanakas transcription factors and green fluorescent protein (GFP) gene reporter in an attempt to reprogramming of cell clone derived from murine melanoma B16F10 cell line into embryonic state. **Methods:** Seven clones were isolated from B16F10 cell line. These clones were characterized by several methods such as immunofluorescence, cell cycle analysis. qPCR for several pluripotent markers expression, inoculation into mice, epigenetic analysis, cell viability by MTT, flow cytometry using a panel of mesenchymal and cancer stem cells markers. One clone was chosen and transfection has been performed using nonviral circular DNA plasmid containing the genes Sox-2, Oct4, Nanog, Lin28 and GFP. The cells isolated were also fully characterized. **Results and Discussion:** Clone 7, which was used for transfection experiments, showed rapid and efficient reprograming in 100% of the cells as evidenced by GFP expression. After 5 days the cells of clone 7 start to present typical morphology of pluripotent stem cells forming islands composed by juxtaposed cells, which also express GFP. This expression indicates the successful reprogramming of clone 7 cells. Interestingly, that after 7 days the GFP expression in reprogrammed cells decreased and at approximately at day 10 no one cells with GFP expression were observed. It noteworthy that these cells, which passed through the reprogramming process die in approximately 15-20 day after transfection. Our data suggest that studied cells were able for reprogramming, however this state was rapidly reversed and even lead to cell die, which was never observed in normal cells. This phenomenon is very exciting and new and surely needs further investigation.

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7.04 A new insight on melanoma cells heterogeneity

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Introduction: Malignant melanoma is a heterogeneous tumor comprised of many subpopulations of cells each with unique genotypic and phenotypic signatures. This cellular and molecular heterogeneity represents a major obstacle to effective cancer treatment and is a basis of drug resistance. **Objectives:** Herein we aimed at isolation and characterization of several cell clones from B16F10 murine melanoma cell in order to evaluate the heterogeneity of prime cell line as well as to test how each these clones will respond to the treatment of several conventional anti-cancer drugs. **Methods:** Clones isolated from B16F10 melanoma cell line were first stained with phalloidin in order to characterize the cytoskeleton of the cells in each clone. Next, clones were characterized in respect of expression of such markers as CD24, CD44, CD19 and CD146 and the progression of these cells through cell cycle was also evaluated all by Flow Cytometry. Western Blot analysis has been performed to detect the expression of such proteins as c-myc, β-catenin, E-cadherin in studied clones. **Results and Discussion:** The cytoskeleton playsan essential role in the proliferation of cells, which has led to the use of drugs that inhibit the cytoskeleton as anti-cancer drugs. The changes in the cytoskeleton organization between different clones was detected as well as the clones demonstrate also significant difference in cell cycle, which also differs from original B16F10 cell line and other controls. These clones were also dissimilar in expression pattern of CD24, CD44, CD19 and CD146 markers, as well as showed significant difference in tumor morphology after inoculation into mice. Interesting that LD50 meaning was individual for each clone. Our data provide new insight on *in vitro* melanoma cells heterogeneity and provide preliminary bases for understanding of intratumoral heterogeneity and drug resistance *in vivo*.

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7.05Monoclonal antibodies from CLNH5 hybridoma recognizes HPV16 proteins

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Introduction: Monoclonal antibodies (mAb)-based products are highly specific for a particular antigen. This characteristic feature of the molecules favors for many applications like cancer diagnosis and therapy. Today, there are fourteen mAb-based drugs approved for the treatment of cancer patients. The CLNH5 hybridoma lineage is descendant of lymphocytes fusion from cervical carcinoma patient, with human cell lineage UC 729-6 that produce IgM monoclonal antibodies. These antibodies can recognize cell lines derived from cervical carcinoma, but do not react with hematopoietic or normal fibroblastic cell lines. High-risk human papillomaviruses (HPVs) are responsible for 99% of cervical cancers and other epithelial cancers. The high-risk HPV16 is the most associated with cervical cancer, it DNA encodes viral proteins L1, L2, E6 and E7 which are responsible for the oncogenic potential. **Objectives:** To produce IgM monoclonal antibodies from CLNH5 hybridoma and verify its interaction with HPV16 E6, E7, L1 and L2 viral proteins, expressed by transfected cells and HPV-positive cells. **Methods:** CLNH5 hybridoma was cultured in RPMI medium with 10% fetal bovine serum. The supernatant was precipitated with saturated ammonium sulfate solution to obtain antibodies, which were assayed in acrylamide gel followed by Western blotting to evaluate the molecular weight and the recognition of anti-human IgM. Also, immunofluorescence (IF) and ultrastructural immunocytochemistry (ICH) with HEK 293T cells transfected with HPV16 *E6, E7, L1* and *L2* genes and CaSki, HeLa and SiHa HPV positive cell lines were done. The morphological analysis of hybridoma was carried out by scanning electron microscopy. **Results and Discussion:** The CLNH5 hybridoma has a spherical morphology with too many plasma membrane projections that increase the contact area with the environment, favoring capture of nutrients, adhesion and locomotion. The IgM antibodies showed molecular weight according to the literature and their interaction with anti-IgM were confirmed by Western blotting. These antibodies were able to recognize HPV16 proteins in transfected cells and in HPV-positive cells by IF and ultrastructural ICH. Antibody-drug conjugates have been developed for targeted delivery of potent anti-cancer drugs to avoid the morbidity common to conventional chemotherapy, and will be the focus of many biotechnological and pharmaceutical programs in the next years, being an important research investment.

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7.06 *Crotalus durissus ruruima* snake venom and a phospholipase A_2 isolated from this venom activate inflammatory response in macrophages

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Introduction: The venom of *Crotalus durissus ruruima* snake, a subspecies found in the cerrado of Roraima, presents lethal, coagulant and myotoxic activities and high concentration of crotoxin. A FLA_2 (CB*r*) with high degree of identity with the FLA_2 from *C. d. terrificus*, that modifies macrophage functions, was isolated from that venom. Upon activation, macrophages display several inflammatory activities, including increase in the number of lipid droplets (LDs). These organelles are relevant for synthesis of inflammatory mediators and their formation is dependent on the protein perilipin 2 (PLIN2), which plays a key role in LDs assembling. However, the effects of the whole venom of *C. d. ruruima* and its FLA_2 in macrophages are unknown. **Objectives:**This study evaluated the effects of VCdr and CBr in macrophages, focusing on: i) formation of LDs, ii) protein expression of PLIN2, iii) subcellular distribution PLIN2 and PGE_2, iv) release of PGE_2, PGD_2 and TXA_2, v) protein expression of COX-1 and -2 and vii) involvement of COX-1 and -2 in formation of LDs. **Methods:**Thioglycolate-elicited macrophages from male Swiss mice (BI-Ethical Committee 896/12; 1019/13) were incubated with RPMI or non-cytotoxic concentrations of CB*r* for 1 to 12 h. LB formation was quantified by staining with osmium tetroxide and analyzed by phase contrast microscopy. PLIN2 and PGE_2 distribution was analyzed by immunofluorescence assay and COX-1, -2 and PLIN2 protein expression by western blotting. The concentration of PGE_2, PGD_2 and TXA_2 were determined by enzyme immunoassays. Inhibition of COX-1 and -2 was assessed by pretreatment of macrophages with compounds valeryl salicylate and etoricoxibe, respectively. **Results and Discussion:** Data demonstrates that macrophages incubated with VCdr or CB*r* caused increase of LDs numbers from 1 to 12 h. Further, VCdr and CBr caused formation of cytoplasmic aggregates of PLIN2 co-located with LDs and PGE_2, and induced a significant increase of PGE_2, PGD_2 and TXA_2 release. In contrast, both VCdr as CBr neither induce protein expression of COX-1 nor COX-2. Pretreatment of macrophages with COX-1 inhibitor significantly decreased formation of LDs induced either by VCdr or CB*r*. In conclusion, the results indicate that both VCdr and CBr are able to directly stimulate macrophages to release of PGE_2, PGD_2 and TXA_2 and to form LDs. Co-localization of PGE_2 with LDsimplicates these organelles as sites for synthesis of PGE_2. Moreover, formation of LDs by these components depends, in part, by activation of COX-1.

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7.07Human Dental Pulp Stem Cells in the Treatment of Atherosclerosis Induced in Rabbits with a Cholesterol Rich Diet

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Introduction: Atherosclerosis is characterized by lesions in the intimal layer called atheromas, which make protrusions in the vessel lumen and weaken the underlying medial layer. The use of intrinsic and extrinsic endothelial progenitors cells (EPC) raise as a possible treatment for vascular repair and regeneration. Nevertheless, mesenchymal stem cells/medicinal signal cells (MSC) showed several advantages over EPCs due to both regenerative and paracrine properties and have been underexplored in this disease as a possible medicinal tool. **Objective:** Evaluate the ability of human immature dental pulp stem cell (hIDPSC) to interfere on morpho-pathological context of atherosclerotic lesions induced in rabbits with 1% cholesterol diet.**Methods:** 14 New Zealand rabbits were divided into two groups: A) Therapy - hIDPSC transplant; B) Control - placebo solution. Each group was separated into two arms: 1) 1% cholesterol diet; 2) Standard diet. After 30 days, $2x10^6$ hIDPSC were transplanted intravenously on weekly applications in the animals A1 and A2, while animals B1 e B2 received saline solution only. The animals were euthanized on day 60, one week after the fourth transplant. The engraftment of hIDPSC was detected using anti-human nucleus (AHN) and anti-hIDPSC antibodies by flow cytometry and immunohistochemistry techniques. The potential effect was evaluated using anti-bodies against human CD34 (EPC marker) and macrophages CD63 and CD14. The expression of vimentin, desmin, alpha actin proteinsand collagen I,were also investigated.**Results and Discussion:** The hIDPSC showed expressive migration with robust engraftment in the intimal layer of the aortic arch in animals A1 in comparison with animals B1 (AHN: ≅39.6%; anti- hIDPSC: ≅26.7%). Unexpectedly, a large number of immunopositive cells for human CD34 antibody was detected (≅23.6%), indicating possible contribution of these cells to the endothelial repair. On the other hand, a great quantity of cells was immunopositive for macrophage human markers (CD63 and CD14: ≅65.2%). Vimentin and alpha actin had a higher expression in animals A1 rather B1, while desmin have been little expressed in these groups, as expected. All proteins were negatives in animals with standard diet (A2 and B2). Since a firmer atherosclerotic plaque could prevent thrombosis to happen,the production of Collagen I were more expressive in the thickened intimal of the animals A1 (≅41.2%) when compared to animals from other groups.

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7.08Presence and location of immature stem cells in human dental pulp

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Introduction: With the current longevity, people have improved the oral health care and to this the regenerative treatment are necessary mainly to create bones where the implants are put. In many situations, regenerative techniques have been improved and developed, and in this context the isolation of immature stem cells from dental pulp (CTIPDs) has been highlighted. These types of stem cells have the origin from the neural crest, and so, it has been demonstrated that these cells have the capacity to differentiate into bone tissues, adipocytes, neurons, glial cells, and other cell types. However, until to now it is not known the exactly location of the niches of these cells in the pulp tissue. **Objective:**Thus, the present study aimed to identify and characterized the presence of CTIPDS in pulp tissues. **Methods:**For this, pulps of permanent and deciduous teeth were evaluated through histopathological protocol, in order to determine some specific histological events. In the same pulps a imunohitochemistry were done with some markers of pluripotency.**Results and Discussion:** The pulps evaluated demonstrated the expression of specific markers of pluripotency and also of CT in particular CD73, CD105, nestin and vimentin. Histologically, the pulps had little or no inflammatory cells, or with normal appearance. Such characterization is crucial, because it opens a wide field of use of CTIPDs. With this we can prove that the pulps obtained was possible to characterize the presence of CTIPDs

Support by Fapesp

7.09Antibacterial activity of snake β-defensin-like polypeptides.

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Introduction: β-defensins are vertebrate innate immune system components; they are small cationic peptides with antiparallel triple-stranded β-sheet and six conserved cysteines with particular spacing and linked 1-5, 2-4, 3-6. They have antimicrobial activity being fully studied in mammals but having little information about them in snakes. β-defensin-like polypeptides have been reported in venoms of sea anemones, snakes and platypus, which display numerous pharmacological effects, including ion-channel inhibition and myonecrosis. We have described β-defensin-like genes in *Bothrops* and *Lachesis* snakes which codify mature peptides (MP) with six conserved cysteines, basic amino acids residues, small size (about 5 kDa), positive net charge and high hydrophobicity. **Objectives:** The main objective of this work was to test the antibacterial activityof recently described β-defensin-like peptides of Brazilian snakes. **Methods**: Synthetic peptides were reduced with dithiothreitol, alkylated with iodoacetamide and purified in a C18 column by gravity. Reduced β-defensin-like peptides were tested against Gram-positive and Gram-negative bacteria from snake bacterial flora and ATCC strains by use of a modified microbroth dilution assay. **Results and Discussion:** We tested 14 linear peptides and a native crotamine. Peptides with net charge greater than +2 showed minimal inhibitory concentration (MIC) against *Micrococcus luteus* ranging from 2 to 128 µg/mL, that with net charge greater than +6 showed inhibitory activities against *Escherichia coli* with MIC from 8 to 128 µg/mL, and that with net charge greater than +7 showed MIC against *Citrobacter freundii* from 8 to 128 µg/ml. Only DefLm02 and DefBm02 peptides showed partial inhibitory activity against *Staphylococcus aureus* at concentrations of 32 µg/mL and 64 µg/mL, respectively.None peptide presented inhibitory activity against *Klebsiella pneumoniae, Morganella morganii, Providencia rettgeri* and *Serratia marcescens.* The native form of crotamine was more active than reduced one. Linear crotasin showed no antimicrobial activity against the tested species. It has been observed that the most cationic peptides (net charge from +6 to +11) showed antimicrobial activity. The results confirm that the positive net charge is the most important biochemical characteristic of β-defensin-like peptides to their antibiotic activity. The high content of positively charged residues in those peptides enables them to interact electrostatically with the negatively charged bacterial membrane.

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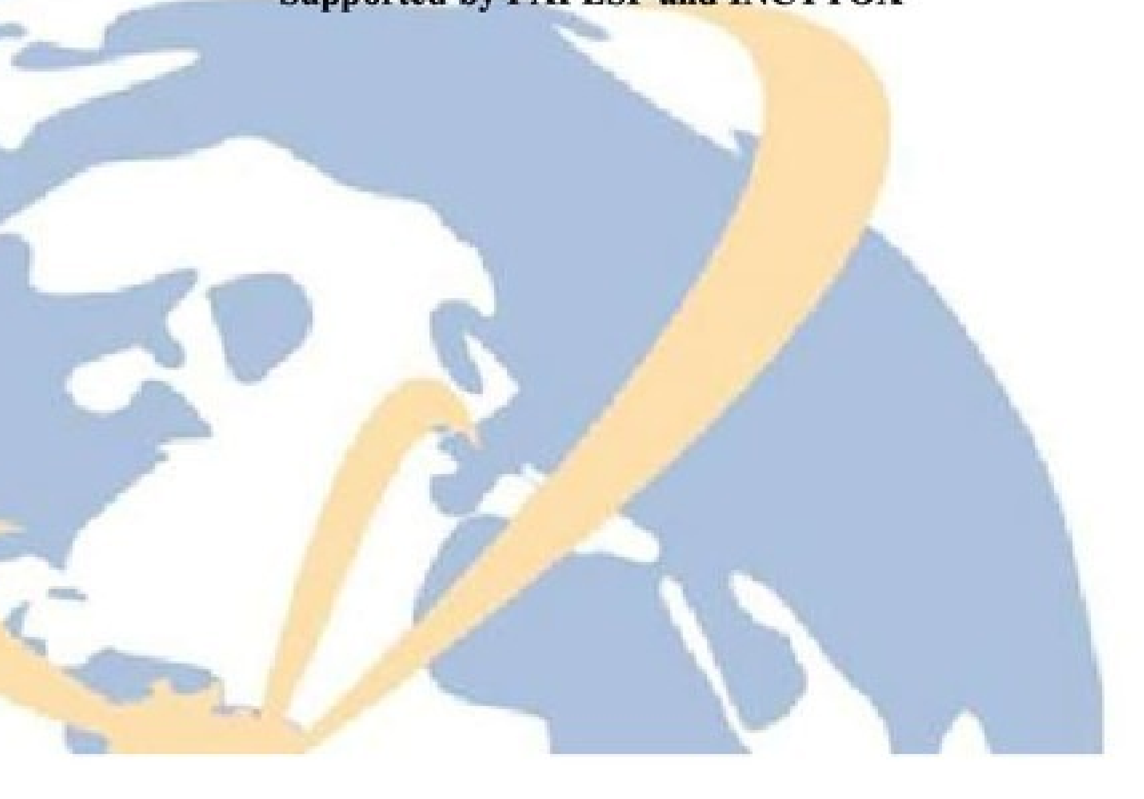

7.10Anticancer activity of copaiba oil, andiroba oil and dexamethasone on oropharynx squamous cell carcinoma line and evaluation of NF-kB expression

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Introduction: Squamous cell carcinoma represents more than 90% of oral cavity malignancies. In advanced cases, when regional control is not possible using only surgery and radiotherapy, adjuvant chemotherapy may play an important role to improve the quality of life and survival of these patients. Molecular events that occur during carcinogenesis are similar to those observed in the inflammatory process. Consequently, the study of anti-inflammatory substances may be an important approach for the development of new chemotherapeutic drugs for cancer. **Objectives***:* The aim of this study was analyze a possible anti-tumoral effect of 2 natural anti-inflammatory oils (Copaiba and Andiroba) and a synthetic drug (dexamethasone) in an oropharynx squamous cell carcinoma line (FaDu). Moreover, correlate NF-kB protein expression with the inhibition of cell proliferation. **Methods***:* Drugs effects were evaluated through quantitative methods (Western Blot, growth curves and cell viability assay) and qualitative analyses (Immunofluorescence and TUNEL-assay) before and after cell treatment. **Results and Discussion***:* All drugs promoted reduction in growth and cell viability but with different potential actions. Copaiba oil, Andiroba oil and dexamethasone presented a potent anti-proliferative action and Copaíba oil induced apoptosis. A decrease expression of NF-kB was confirmed by western blot and immunofluorescence, after treatment with the three substances. Copaiba and Andiroba oils and dexamethasone promoted inhibition of cell proliferation, showing an association with decrease expression and inactivation of NF-kB, which regulates genes important in proliferation and cell survival.

7.11Cytotoxic and anti-proliferative effects of crotamine in normal and cancer cell lines

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Introduction: Crotamine (CROT) is a major component of rattlesnake (*Crotalus durissus terrificus*) venom. This is a small non-enzymatic basic polypeptide that once inoculated on host, causes hind limbs paralysis and extensive necrosis of muscle cells. On other the hand, CROT at low concentrations is uptake by proliferating cells via endocytosis mediated by binding to cell membrane heparan sulfate proteoglycan. We verified thatCROT exhibits cytotoxic effect against mouse and human melanoma cells *in vitro* besides inhibiting tumor growth *in vivo*. **Objectives:** to investigate the mechanisms involved in CROT cytotoxicity in normal and cancer cell. **Methods:**The IC50 was determined by using MTT test. The cytotoxic effect, the cell cycle modulation and on mitochondria potential was evaluated through cytometry and confocal microscopy after CROT treatment. Nuclear and cytoplasmic morphologic changes were also accessed by light and fluorescence microscopy. **Results and Discussion:** CROT exhibits a selective toxicity mainly in high proliferating cells (cancer cells). Beyond this, CROT collapses the cytoskeleton structure of cancer cells triggering apoptosis. Further, our findingindicates that CROT exhibits pronounced and selective effect in melanoma cells as compared with others cancer cells. More studies need to be performed to elucidate the CROT cytotoxic mechanisms. CROT have demonstrating cytotoxic effects in a broad range of cancer cell lines. Interesting we found that the CROT cytotoxicity is directly proportional to the rate of cell proliferation. Thus, cytotoxicity was induced in cells high proliferative melanoma cell lines while a discrete cytotoxic effect was induced in normal cells (CHO). This differential effect can be attributed at least in part by the turnover of surface proteoglycan such as heparan sulfate.

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7.12Studies on the expression of the*Human papillomavirus* (HPV) in Peripheral Blood, Retinoblastoma and Cervical Samples

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Introduction: The Human Papillomavirus (HPV) has been extensively studied due to its carcinogenic potential, related to the integration of viral DNA into the host genome. About 120 different types of HPV have already been described based on the heterogeneity of genome. According to the frequencies of viral diagnosis associated with carcinoma cases, these viruses were classified as low, intermediate or high risk. Among the high-risk types, also called oncogenic, the most common is HPV-16, accounting for about 60-70% cases of cervical cancer associated with viruses. In addition to carcinoma of the cervix, HPV has been linked to several cancers, including retinoblastoma. **Objectives:** This study aims to evaluate the activity of HPV in cervical material, peripheral blood and samples from child retinoblastoma. **Methods:** Samples from 34 women and 2 children were collected. DNA was extracted from all samples and from peripheral lymphocytes Obtained from short term lymphocyte cultures. Viral detection was performed by PCR with generic and specific primers for the HPV-16 and 18. Immunohistochemistry and comet assay were performed in order to verify viral presence in affected tissue and clastogenic viral action. **Results and Discussion:** HPV was detected in 29 (85.3%) women and two children with retinoblastoma, HPV-16 being the most frequently found. By immunohistochemistry it was possible to demonstrate the viral proteins E1 + E4 (anti-HPV-16) and L1 (anti-HPV 1, 6, 11, 16, 18 and 31) in paraffin-embedded tissue in different regions of the eye, confirming viral activity in the sample. Furthermore, DNA damage in women with HPV were shown by comet assay, and significant statistic differences were found between lymphocytes 48h cultured of patients positive for HPV compared with those of women negative for the virus ($p = 0.019$). It is assumed that the damage observed in the host's DNA is a result of viral activity and presence.

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7.13Multiple pathways of HPV16 VLP uptake in peripheral blood mononuclear cells

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Introduction: Human Papillomavirus (HPV) is an intracellular virus which need a host cell to express their proteins and to replicate. To this, the HPV's journey from extracellular space to specific sites is composed of successive steps which start with the interaction and internalization of this pathogen. Several studies describe this process like a model complex of interactions of different pathways, receptors, co-receptors and co-factors. In keratinocytes, HPV seems to be internalized via clathrin-dependent endocytic mechanisms, but it maybe uses alternative uptake pathways to enter cells, such as a caveolae-dependent route and others. However, the plasticity of many cellular pathways means that viral entry may be impacted by an indirect mechanism rather than by direct inhibition. **Objectives:** To study different membrane receptors and internalization pathways for HPV16 VLP L1L2 in peripheral blood mononuclear cells (PBMC). **Methods:** Blood of healthy donors were collected with heparin and the PBMC were isolated, counted and incubated in RPMI medium overnight over coverslips containing poly-L-lysine, at 37°C and 5% CO_2. Then, the cells were washed and a fresh medium was placed together with specific biochemical inhibitors of ligand uptake (chlorpromazine, *Clostridium* toxin B, filipin, nystatin, liquemin), used isolated or associated, and incubated for 2 hours. After incubation, the cells were washed and HPV16 VLP L1L2 were added and incubated with medium for 4 hours. In the next step, the cells were washed again and fixed in paraformaldehyde. Immunofluorescence assays were carried out to detect the VLP-PBMC interaction by confocal microscopy. **Results and Discussion:** The global concern is that HPV DNA has been detected in extra oral-genital tumors such as breast, lungs and non-melanoma skin. In these cases, the HPV does not appear to be responsible for carcinogenesis, but contributing factor to tumor development due to the pre-existing genomic instability. The use of different biochemical inhibitors isolated or associated seems not be enough to block the entry of HPV16 VLP L1L2 in the PBMC, suggesting that a specific receptor is not required to the uptake and HPV16 entry into the host cells, and that once reached the bloodstream, the virus may be spread to whole body inside PMBC,contributing for malignancy development. These findings suggest that HPV16makes use of multiple internalization pathways to infect host cells.

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7.14 Effects of Jararhagin-C, a disintegrin-like molecule from *Bothrops jararaca* venom, on wound healing process.

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Introduction: There are a number of specific integrins that anchor endothelial cells to extracellular matrix components, modulating events during angiogenesis and wound healing process. Jararhagin-C is a disintegrin-like molecule containing an ECD sequence isolated from *Bothrops jararaca* venom which can bind to α2β1 integrin present on platelets membranes. Endothelial cells in turn express different integrins on its surface according to their environment. **Objectives:** This study aimed to investigate the effects of jararhagin-C on human vascular endothelial cells (HUVEC) during initial events related to wound healing process. **Methods:** Firstly it was verified thebinding capacity of jararhagin-C on HUVECsthrough inhibition of cell adhesion assay. Suspensions of HUVECs were incubated with jararhagin-C and seeded on well plates pre-coated with collagen I, IV or fibronectin. It was observed a slight decrease in adhesion cells to collagen I, suggesting that jararhagin-C bound itself on HUVEC. The cell migration assay was performed on HUVECs monolayer at an *in vitro* experimental model of wound healing and analyzed by optical microscope. This molecule induced cell migration on collagen I and IV surfaces. The jararhagin-C proliferative activity was evaluated on HUVECs cultivated on collagen I, IV or fibronectin substrate. The proliferation assay was performed by BrdU method. Jararhagin-C does not interfere in the proliferative capacity of these cells. Finally we analyzed the time-course of gene expression of 9 different genes involved in the wound healing process. This experiment was evaluated by Real-time PCR on HUVECs previously adhered to collagen treated by jararhagin-C and compared with a control group of cells treated with PBS. The fold change (Fc) was calculated by ΔΔct equation. **Results and Discussion:**At the time interval of 3 hours we observed up-regulation of IL-6, CXCL-6, and MMP-10 (Fc = 3.0; 1.8 and 2.5 respectively), At 6 hours the genes E-Selectin, I-CAM-1, IL-8, Angiopoetin-2 and MMP-10 were up-regulated (FC = 6.0; 7.0; 2.0; 2.5 and 2.0 respectively) while IL-6 was down regulated (Fc = -0.5), At 24 hours we observed down regulation of IL-8, CXCL-6 and CD-69 (Fc= -0.5; -0.3 and -0.4 respectively). Our results suggest that jararhagin-C can bind to endothelial cells growing on collagen I surface. Jararhagin-C can modulate HUVEC migration on collagen I and IV surfaces. However this molecule by itself is not able to induce endothelial cells proliferation on these substrates. Jararhagin-C induces up-regulation of genes expressed by endothelial cells involved with wound healing process. The jararhagin-C ability to bind to integrin α2β1 is probably the responsible by the effects observed here.

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7.15 Investigation of histones post-translational modifications in adrenocortical tumor cells stimulated with fibroblast growth factor 2

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Introduction: Post- translational modifications (PTM) of histone tails by phosphorylation, methylation and acetylation can be related to chromatin regulation, gene expression and cell cycle forming the so called "histone code". It is known that growth factors can induce cell cycle progression promoting alteration on histone PTMs. The fibroblast growth factor 2 (FGF2) is associated with proliferation and carcinogenesis but has anti-proliferative and tumor suppressive functions on some cellular contexts. In Y1 murine adrenocortical carcinoma cell line, the FGF2 promotes G0/G1 transition but delays S-phase and permanently block cells in G2/M leading to the appearance of senescent phenotype cells. **Objectives**: It is not known if FGF-2 can affect histone PTM dynamics on Y1 cells and also if it can play a role on cell cycle blockage and senescence induced by FGF-2. Thus, the main goal of this project is to investigate the dynamics of histone PTMs after FGF2 stimulation on Y1 cells along cell cycle and senescence. **Methods:** Y1 cells were plated in cells dishes (9 x 10^3 cells per cm2) and cultivated until reaching 30% of confluence when fetal bovine serum (FBS) was removed. After 48h, the cells were stimulated with DMEM supplemented with 10% of FBS or DMEM with 10% FBS and 10 ng/ml of FGF-2 for 0h, 15 min, 30 min, 1h, 3h, 5h and 12h. Cells were harvested and lysed on Triton Extraction Buffer (PBS containing 0.5% Triton X-100 + protease/phosphatase/deacetylase inhibitors) and histones were extracted in 0.2 M HCl. Five micrograms of each timepoint sample were fractionated on SDS-PAGE gel and transferred to PVDFmembranes. Western Blotting assays were performed by using commercial antibodies against H3K4me, H3K4me2, H3K9me, H3K27ac, H3K9me3 and H3K27ac. **Results and Discussion:** Neither FGF-2 nor FBS seems to affect the global level of H3K4me, H3K4me2 and H3K9me3 after stimulation. The PTMs dynamics of H3K9me, H3K27ac and H3K27me3 evaluated by western blotting are still inconclusive. Therefore we intend to work on the establishment of enzyme-linked immunosorbent assay performed on nucleosomes (NU-ELISA) to quickly and effectively quantitate global levels of histone PTM after FGF-2 stimulation.

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7.16Establishment of primary cultures of cancer stem cells for studying humanLi-Fraumeni Syndrome

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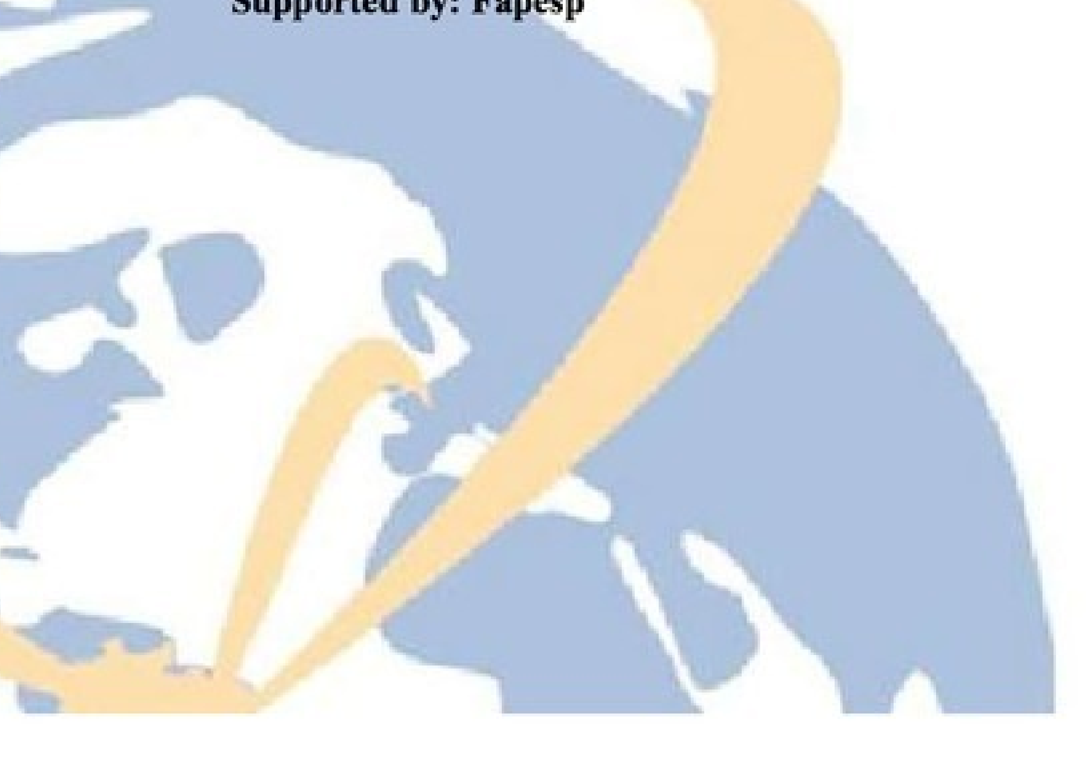
Introduction: Li-Fraumeni syndrome (LFS), an inherited cancer predisposition syndrome, is associated with germline mutations in *TP53*. It is characterized by high risk of multiple, early cancers. In Brazil, a variant form of LFS is exceedingly frequent due to a widespread founder *TP53* mutation, p.R337H, detected in about 0.3% of the general population in Southern Brazil. This mutation occurs in p53 oligomerization domain and its effect on p53 oligomerization is supposed to be dependent upon pH conditions. Recent studies indicate that p53 plays a critical role in regulating differentiation and asymmetric divisions of stem cells. **Objectives:** The main objective of this study is to isolate and to characterize Cancer Stem Cells (CSC) and Mesenchymal normal stem cells in patients with germline mutations in TP53 gene with Li-Fraumeni syndrome and Li-Fraumeni-*like* Syndrome. **Methods:** We have isolated and characterized CSC from tumors of p.R337H mutation carriers. After informed consent, surgical resection fragments were dissociated and brought in culture. Adherent cells and spheroids were derived from different tumor types. Spheroids and stromal cells derived from a breast cancer (BC) were further analyzed by immunofluorescence and flow cytometry to demonstrate positive immunolabeling for $CD44^+$, $CD24^-$, Oct4, Ki67, Sox2 and P53 antibodies.**Results and Discussion:** Time-lapse videomicroscopy showed rapid growth, frequent asymmetric division and absence of senescent phenotypes for least 17 passages. Moreover, we showed by Colony Forming Units assay (CFU) that stromal cells are very clonogenic, once 10^3 cells initially plated were able to form 442 new colonies after 15 days in culture. It was also verified the differentiation potential of stromal cells for the following lineages: adipogenic, osteogenic and muscle-like cells. The adherent stromal cells as well as oncoesferas are able to self-renew and to proliferate in vitro for long periods without losing its main features. These properties are similar to stem cell possessing the p53 inactive. However, we observed that treatment of adherent cells with doxorubicin (DNA damaging agent) causes accumulation of p53 and causing induction of cell cycle arrest with low doses of this drug, and cell death / apoptosis in higher doses. Our findings using CSC-p.R337H can provide a very useful model for better understanding the role of specific mutation, as well as the TP53 tumor biology.

Supported by: Fapesp



7.17 The potential of eugenol as anticancer agent

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Introduction: Cancer is a leading cause of death in industrialized countries consisting in a group of diseases characterized by several features acquired by malignant cell outlined hallmarks of cancer such as self-sufficiency in cell signal, insensivity to antigrowth cell signal, tissue invasion and metastasis, limitless replicative potential, sustaining angiogenesis and evasion of apoptosis. Thus, the search for molecules with therapeutic potential against cancer as well as elucidating its mechanism of action is crucial issue to reduce the amount of dead related to cancer cell. Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) is an essential oil found in several spices of plants such as *Syzgium aromaticum* (clove), *Pimenta racemosa* (bay leaves), and *Cinnamomum verum* (cinnamon leaf). Among these, the essential oil extracted from the clove plant presents a higher content in eugenol (72-90%). Therapeutics appliance attributed to eugenol include analgesic, antibacterial, antifungal, anthelmintic, antiplasmodial, antiviral activity can be used in cases of gastrointestinal diseases and chronic diarrhea, but the major usage is still in the dentistry as an analgesic and local anhestesic. Futhermore, eugenol has been demonstrating antiproliferative properties against a broad range of cancer. **Objectives:** To investigate the cytotoxic potential of eugenol in melanoma and breast cancer cell lines. **Methods:** Melanoma and breast cell lines were cultured until reached confluence, after adding different concentrations of eugenol to the culture, the MTT assay was performed. The scanning electron microscope (SEM) technic was applied to investigate the ultrastructure changes. Finally, caspase-3 activation was evaluated by flow cytometry.**Results and Discussion:** Herein, we demonstrated that eugenol exhibits cytotoxic activity in the cells (SKBR3, B16F10, A2058, and Mel85) from MTT assay; In addition, eugenol was capable to cause deeply morphologic changes in these cells, suggesting apoptosis as way of cell death. Flow cytometry was performed in order to confirm the caspase-3 activation corroborating apoptosis. Thus we suggest that eugenol acts as apoptotic agents with interesting anticancer properties.

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7.18Evidence for the expression of bovine papillomavirus in lines primary of the epithelial cells infected with BPV.

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Introduction: Bovine Papillomavirus (BPV) is an epithelium oncogenic DNA virus, specie and tissue specific. Studies have reported the virus genome presence in other non-epithelial tissues, and recently many evidences expression of these DNA sequences have discussed their infection potential. One of them is the high level of chromosomal abnormalities found in lymphocytes from chronically infected animals and the presence of virus DNA in fibroblast cells *in vivo* and *in vitro* cultures. **Objectives:** To evaluate the expression of the BPV in primary cell lines obtained from infected lesions with BPV and to verify the levels of clastogenicity in these cells, comparing this clastogenicity with peripheral blood in the animal affected with BPV. **Methods:** Comet assay was performed in primary cell lines of cutaneous papillomas, esophagus, bladder and lymphocytes from peripheral blood of bovines with papillomatose and bovines without papillomatose (control). *DNA virus detection*:DNA extraction of cells was performedusing *mini KitTissue Protocol* – Qiamp DNA and PCR reactions used Master Mix. The molecular identification was performed using especific primers BPV-1/2 and 4 to amplify fragments of the L1 gene. *Immunofluorescence*:Was used for cell labeling monoclonal antibody to papillomavirus (PV) [L1 (HPV), E2 (BPV-1), E1+E4 (HPV-16) and E7 (HPV-18)]. **Results and Discussion:** The primary cell lines and blood were positive for BPV-1 and -2. Besides was imuno-positive to fourth proteins of the PV: L1, E2, E1+E4 and E7. Higher levels of clastogenicity in primary cell lines were evidenced. It was observed a significant increase ($p<0.05$) of the clastogenicity in cutaneous papillomas cell passages (P1-P6). Also was significant the increase of the clastogenicity in lymphocytes peripheral blood ($p<0.05$). These evidences point out that virus genome sequences found in the cultures are active, causing damage in host chromatin. Further analyzes on viral load and expression of BPV oncoproteins will be conducted to elucidated this aspect. Furthermore, comparisons between the results of viral protein expression in the different sample sites (blood, primary cell lines of the papillomas) will be performed.

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7. 19Intracellular peptides analysis in cells HeLa expressing the immune proteasome: possible correlations to cell signaling

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Introduction: Proteasomes are multisubunit multicatalytic proteases that are responsible for the majority of nonlysossomal protein degradation within eukaryotic cells. The 20S catalytic core is composed of 28 subunits assembled in four stacked seven-membered rings. The outer rings contain seven different non-catalytic α-type subunits, and the inner rings contain seven different β-type subunits, three of which are catalytic (beta1, beta2 and beta5). Under conditions of stress or immune response, these three subunits may be replaced by β1i, beta2i and/or beta5i to form the immune proteasome, which has different catalytic specificities of the constitutive proteasome. The inducible subunits appear to be responsible for altered peptidase specificities in IFN-y-treated cells. Moreover, it is known that this modification provides a better antigen presentation via MHC class I. **Objectives:** Our aim in this work is to characterize the intracellular peptide content after immune proteasome induction.**Methods:** HeLa cellswere grown understandard cultureconditionsuntil60% confluence and subsequently incubated with (200U) or without INF-y for 48 hours. After confirmation of the immune proteasome expression by Western Blotting using specific antibodies against subunits beta5 and beta5i, peptides extracted from control and experimental group were quantified using fluorescamine and then labeled with light isotopes (D0-TMAB) or heavy isotopes (D3-TMAB). The semi-quantitative analysis and identification of peptide sequences were performed mass spectrometer followed up by searches in the NCBI database using the MASCOT software. **Results and Discussion:** A total of 85peptides wereidentified, all derived from intracellular proteins(nuclear, mitochondrial and cytosolic). Only one of thesepeptides, named EL28,appear significantlyincreased inHeLacellsstimulated withIFN-y comparedwith controls,suggesting that the immuneproteasome induction only changes specificintracellularpeptides.Proteasome activity assay performed after in vitro treatment with the peptide showed an increase in chymotrypsin-like activity compared to control.EL28 could be of clinical relevance for treating disorders related to antigen presentation as it can target to increase the chymotryptic activity of the proteasome.

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7.20C-terminus of protein S100A9 inhibits events involved in *in vitro* angiogenesis

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Introduction: Our group has showed that both S100A9 and a synthetic peptide identical to the terminal region of this protein (mS100A9p) induce antinociceptive effect. In addition, the mS100A9p inhibits mice adherent peritoneal cells functions, cells critical for interactions with both malignant and stromal cells in the local microenvironment. Literature data shows that the complex S100A8/A9 is being associated to malignancy.In cancers, the proliferative state is accompanied by intense angiogenesis, a process that evolves new vase formation and cell migration. However, studies focusing the effect of the mS100A9p on tumor progression and angiogenesis have not been carried out yet. **Objectives:**the aim of the present study was to evaluate the *in vitro* effect of the mS100A9p on events involving in angiogenesis. **Methods:** Proliferation assay:$5x10^4$ thymic endothelial cells(tEnd.1) were incubated in a 6 well plate for 1h with culture medium (control) or culture medium containing different peptide concentrations (0.585; 1.17; 2.35; 4.7 or 9.4μM/well). Then it was washed and after 24h incubation with RPMI, the cells were stained with Trypan blue and cells number determined in Neubauer Chamber. Cell migration assay (*Wound healing*):$1x10^6$ tEnd.1 cellswere plated in a 24 well plate and after became confluent a wound was made with a sterile tip. Cells were incubated with only RPMI 1640 medium at 10% bovine fetal serumor mS100A9p, on the same concentrations mentioned, before for 1h. It was washed with PBS and left for 24h with RPMI 1640 1%. After this period were counted the cells which migrated to the wound. Adhesion assay:96 well plate were sensitized with extracellular matrix component (fibronectin 3μg/well) for 16h. Then, it was incubated for 1h with 1% BSA and after it $5x10^5$ tEnd.1 cells/well treated or not with mS100A9p (2.35μM concentration) for 1 hour and added to the plate for another hour. Adhered cells were incubated with MTT for 3h and then with SDS for 18h and read in ELISA (Multiskan EX, Labsystem). **Results and Discussion:** It was found that all concentrations inhibited cell proliferation and migration, but 2.35μM was the more effective (37% and 56%, respectively), in relation to control group. This concentration also inhibited (21%) cellular adhesion to fibronectin. All together it shows that mS100A9 peptide is capable of modulating *in vitro* events involving angiogenesis. These data suggesting that besides inhibiting inflammatory cells function, the peptide interferes with neovascularization development.

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7.21The selective uptake of a novel proteasome inhibitor by tumor cells

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Introduction: Amblyomin-X is a TFPI-like inhibitor that was identified through the transcriptome analysis of the salivary gland from the adult *Amblyomma cajennense* tick. The recombinant form of this protein is also able to induce cell cycle arrest and apoptosis in different tumor cell lines, and promotes regression of tumor growth and reduction of metastasis. The pro-apoptotic stimulus of Amblyomin-X involves mainly proteasome inhibition, endoplasmic reticulum stress,production of reactive oxygen species and intrinsic apoptosis pathway activation. Interestingly, any of these effects were found in non-tumor cells. **Objective:** Inquire if the Amblyomin-X selectivity by tumor cells is associated to uptake of this molecule. **Methods:***Amblyomin-X labeling:* Amblyomin-X was directly labeled with the amine-reactive dye through a commercial kit and was called 488-Amblyomin-X. *Cellular uptake studies:* Human fibroblasts andSK-Mel-28 and Mia-PaCa-2 tumor cell lines were cultured and treated with 488-Amblyomin-X and observed under confocal microscopy. The same procedure was performed with a pretreatment of some endocytosis inhibitors. Besides, cell lysates from this sample were evaluate by fluorimetry and mass spectrometry.*Microinjection:* Amblyomin-X was injected into the cytoplasm of human fibroblasts and apoptosis was scored based on morphology and Hoechst staining. *Proteasome co-localization*: Tumor cell lines were treated with 488-Amblyomin-X and then cells were fixed, permeabilized and incubated with the appropriate antibodies.The slides were mounted and visualized by confocal microscopy. **Results and Discussion:** 488-Amblyomin-X fluorescence signal was detected only in the tumor cells line and was affected by endocytosis inhibitor, collaborating with this finding no intact protein was found in the whole lysate from cells treated with 488-Amblyomin-X. When microinjected into the fibroblasts, Amblyomin-X triggers apoptosis. Furthermore, the recombinant labeled protein was able to co-localize in the 20S proteasome. Taken together, beyond suggesting that the internalization of Amblyomin-X could be regulated by membrane trafficking and endosomal pathway, this finding proposes that cell membrane could be crucial regarding the pro-apoptotic effects of Amblyomin-X, as proteasome inhibition, and that there are differences tumor and non-tumor cell surface, that are relevant for understanding cancer disease as well as development of new drugs selective for certain tumors.

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7.22Blockage of DNA replication in *T. cruzi*: a possible role for RPA-1-telomere interaction

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Introduction:*T. cruzi* is the etiologic agent of Chaga´s disease, a tropical diseases that causes 40,000 new infections per year. Improving the knowledge about the molecular biology of this parasite may facilitate the discovery of new therapies and antiparasitic drugs. Although the biology of *T. cruzi*has being studied over a century, the molecular bases that impair DNA replication in infective forms remain unclear. Telomeres are formed by the interaction of DNA with protein complexes which are responsible for maintaining these terminals. Replication Protein A (RPA) comprises a trimeric complex formed by three subunits, that performs, alone or together with other proteins, various vital functions in DNA metabolism, such as replication, repair and telomere maintenance. **Objectives:**This study aims to characterize the *T. cruzi* RPA-1 and its role in telomeres. **Methods:** After cloning and expression recombinant RPA-1, we perform Circular Dichroism, electrophoresis mobility shift assays (EMSA), Imunofluorescence (IF), Chromatin Imunoprecipitation (ChIP), to study interaction of these molecules with telomeric DNA *in vivo* and *in vitro*. **Resultsand Discussion:** We have cloned and purified rTcRPA-1 that is in correctly fold as analysed by Circular Dichorism. Antibodies were produced against this protein. *In vitro*, we showed that rTcRPA-1 can interact with telomeric G and C rich single strand, but do not with double strand telomeric DNA. *In vivo,*TcRPA-1 is a nuclear protein and interacts with telomeric G strand in G1/S phases of the cell cycle, but not in G2 in epimastigotes, suggesting that this protein is involved in replication of telomeres in this lifeform. We performed ChIP assays in tripomastigote and showed that TcRPA-1 interacts with telomeric G strand too. Based on these evidences, we are investigating the role of RPA-1 in *T. cruzi* telomeres, since tripomastigotes are non-replicative forms. We are working on the hypothesis that RPA-1 is involved in a repair pathway in tripomastigotes. To analyse this possibility we showed that telomere length is shorter in tripomastigotes, compared with epimastigotes. Therefore we are proposing that the shorter telomeric region in tripomastigotes trigger a repair pathway using RPA-1 that might be involved with the lack of DNA replication in non-replicative *T. cruzi* lifeforms. This work possibly present an unclear pathway involved with the lack of replication in *T. cruzi*. This pathway might be elucidated in order to develop drugs that can block proliferation of this parasite.

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7.23ATPase activity of Orc1/Cdc6 as a target for drug design against *Trypanosoma brucei*

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Introduction: DNA replication starts with the formation of the pre-replication complex (pre-RC) into DNA regions known as origins of replication. Different from yeast, trypanosomes don't have Cdc6 and Cdt1 in its pre-RC, but only a protein homologous of Orc1 and Cdc6 named Orc1/Cdc6, which interacts with the MCM helicase, licensing origins of replication. Our laboratory has demonstrated that the *Trypanosoma cruzi* (etiological agent of Chaga's disease) and *Trypanosoma brucei* (etiological agent of Sleeping sickness) recombinant proteins Orc1/Cdc6 bind and hydrolyze ATP *in vitro* and that the ATPase activity increases in the presence of nonspecific DNA. **Objectives**: The objective of this study is to evaluate the importance of ATP hydrolysis by Orc1/Cdc6 for the formation and stability of pre-replication machinery in the genome, and so identify a possible target for drug design against trypanosomes**. Methods:** We produced recombinant *T. brucei* Orc1/Cdc6 protein mutated at sensor 2 region (TbOrc1/Cdc6R251,252E) and confirmed the loss of the Orc1/Cdc6 ability to hydrolyze ATP . This gene was cloned into transfection vector. *T. brucei* was transfected and the expression of mutated protein was induced by the addition of 4 μg /ml doxycycline. **Results and Discussion:**TbOrc1/Cdc6 tridimensional structure was obtained by computational modeling and exhibited a conserved architecture with winged-helix domains for interaction with DNA and with sub-domains for ATP binding and ATP hydrolisis.Mutation in the sensor 2 region of *T.brucei* (TbOrc1/Cdc6R/251, 252E*)* drastically reduced ATPase activity compared with wild type TbOrc1/Cdc6 in *in vitro* assay. *T. brucei* procyclic forms expressing TbOrc1/Cdc6R251,252E showed a sharp decline in growth, which evidenced that Orc1/Cdc6 ATPase domain is a goodcandidate to be validated as a target for drug design against*T.brucei.* According to this data, we are seeking molecules composed by nucleotide and peptide. These molecules might discriminate among ATPases from the parasite and the mammalian host and then *act in a specific manner* to inhibit proliferation of trypanosomes, that is the etiological agent of a very known neglected disease.

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7.24HPV16 capsid proteins expression in human cell line for vaccine formulation

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Introduction: Human Papillomavirus (HPV)is one of the most common sexually transmitted pathogen worldwide. High-risk HPV infection is responsible for approximately 99% of cervical cancers and has been associated with other types of cancer in lower prevalence. The HPV16 is the most prevalent genotype worldwide, being responsible for approximately 50% of cervical tumor cases. Currently HPV vaccine is based on the major capsid protein L1 that can self- assembly into virus-like particles (VLPs), which induce high response against specific-types of HPV. An alternative to VLP vaccines may be of immunization against the minor capsid protein L2, which contains highly conserved sequences that can cross-neutralize a wide HPV types. **Objectives:** The aim of this study is to express HPV16L1 and L2 capsid proteins in human epithelial cells grown in suspension, for vaccine formulation, in order to evaluate immunological responses in mice. **Methods:** Human embryonic kidney (HEK 293F) cells were cultivated in serum-free medium and cells in suspension were incubated at 37ºC with 5% CO_2, under agitation on an orbital shaker. The cells were co-transfected with vectors pUF3/L1h and pUF3/L2h, containing full sequence of *L1* and *L2* viral genes from HPV16, respectively. Transfected and non-transfected cells were analyzed by Western-blotting, confocal and transmission electron microscopies. **Results and Discussion:** In this study, transfected human epithelial cells in suspension expressed L1 and L2 proteins detected by morphological and molecular procedures. Proteins were detected in the cell nucleus and cytoplasm in accordance with previous findings. We have successfully expressed HPV16 L1 and L2 proteins in suspension cells using serum free medium and orbital shaker technology. These results represent an important step for the development of a prototype prophylactic vaccine with a broad spectrum protection against HPV, to be assayed in animal model.

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7.25Antiangiogenic activity of a TFPI-like inhibitor on endothelial progenitors cells

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Introduction: Amblyomin-X is a recombinant protein expressed in *E. coli* system from salivary glands of *Amblyomma cajennense* ticks. This molecule has shown an interesting potential as antitumor agent, inhibiting proteasome, inducing the cycle cell arrest, and leading the tumor cells death by a selective apoptotic process. The inhibition of angiogenesis is considered another important strategy to control some tumor progression. Endothelial Progenitors Cells (EPC) present in human umbilical cord blood are able to differentiate into blood vessels, providing an excellent model to investigate angiogenesis. In adittion, EPC are chemoattracted by the tumor and contribute to intratumor vessel formation.**Objectives:** Evaluate the proliferation of Renal carcinoma cells (RENCA) and EPC *in vitro* and also evaluate matrigel tube-forming assay by EPC after treatment with Amblyomin-X.**Methods:** Proliferation assays were performed for 24 hours in EPC and RENCA (positive control of antiproliferative effect of Amblyomin-X) by acid phosphatase assay (spectrophotometry).**Results and Discussion:**After treatment with Amblyomin-X in a concentration range (0, 0.5, 1, 5 and 10 μM). Amblyomin-X decreased the proliferation of RENCA, particulary, at 5 and 10 μM concentration, but it did not cause any significant changes in EPC growth. However, at the same Amblyomin-X concentration range, the formation of tubule-like structures on a matrigel plate was significantly inhibited. The concentration range of Amblyomin-X able to reduce the proliferation of tumor cells, did not alter EPC proliferation, but it was able to inhibit the formation of tubule-like structures. Taken together, our results indicate that Ambyomin-X presents antiangiogenic effect on EPC.

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7.26 Immunoprofile of porcine adipose-derived mesenchymal stem cells at early and late passages

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Introduction: Adipose-derived porcine mesenchymal stem cells (AT-MSC) have been isolated previously from bone marrow and adipose tissue. These cells were able to differentiate into bone, adipose and cartilage cells however muscle and cardiogenic potential have not been demonstrated. The pigs are the best model to be used in preclinical studies to test regeneration potential and paracrine effect of mesenchymal stem cell (MSC), before their use in humans. Therefore, isolation of porcine AT-MSC (pAT-MSC) is important in order to test the use of autologous cells in cardiovascular diseases. However, these cells were only marginally characterized *in vitro*. **Objectives:** We aimed at *in vitro* isolation and characterization of pAT-MSC, using a wide panel of markers at early and late passages as well as focusing on their muscle and cardiomyocytes *in vitro* differentiation. **Methods:** Adipose tissue fragments were used for isolating stem cells (SC) from healthy adult pigs. The cells isolation was performed as previously described for human ASC by Zuk et al., 2001. Isolated cells were characterized at early and late passages, using wide range stem cell markers. *In vitro* differentiation and teratogenic potential of these cells were also tested. **Results andDiscussion:** At early passages (<P4) fibroblasts-like cells were isolated, which express such markers, as Oct-3/4 (~80%), Sox2 (~79%), Nanog (~35%), CD44 (89%), CD90 (87%), CD105 (95%), vimentin, fibronectin, nestin, pan-cytokeratin (~20%), e-cadherin (~40%), and conexin-43. While at late passages (>P8), immunoprofile of isolated cells was changed: e.g. the number of Oct-3/4 (20%), CD44 (8.7%), vimentin (6.6%) and e-cadherin (28.47%) cells decreased. The expression of pan-citokeratin was not observed anymore. At early passages these cells demonstrated robust differentiation toward bone, cartilage and adipose lineages. After induction of myogenic differentiation the pAT-MSCs showed morphology similar to cardiomyocytes, while expressing cardiotin, specific cardiomyocytes marker and others. After the injection of 10^6 (early passages) pAT-MSC into BALB/c Nude mice, the teratomas formation was not evidenced. Surprisingly, at early passages high number of Oct3/4 positive cells was observed which demonstrate appropriate nuclear localization. Number of stem cells, which express markers of stem cells of epithelial and pluripotent cell type decreased with passages. Porcine AT-MSC of fibroblast-like morphology at early passages was not able to form teratomas, even presenting a high number of the cells, which expressed pluripotent markers within population used in teratoma assay.

Supported by: Capes

8. Animal Biology

8.01Can a fang lost event change the predatory behavior on viperids?

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Introduction:*Bothrops moojeni* (Hoge, 1966) is a viviparous viperid snake of epidemiological importance in Brazil. It is found mostly in Cerrado region, riparian forests, particularly in flooded areas. **Objectives:** We report predatory and feeding behavioral changes in a captive adult female fangless *B. moojeni*, time of prey ingestion and total digestion period. **Methods:** One adult female *B. moojeni* caught in São Simão-SP, Brazil, kept in captivity in the Laboratório de Herpetologia, Instituto Butantan, since 2003 that lost its fangs definitely in 2010. **Results and Discussion:** During the regular monthly venom milking activities in the Laboratory it was possible to observe that usual periodic fang replacement stopped in a female *B. moojeni*. Since then the snake could not kill live prey offered despite their venom production remained. Soon after the fangs lost event, the snake still displayed the typical viperid stroke behavior but prey showed only wounds caused by maxillary and mandibular teeth and were not swallowed. So previous killed mice and rats (warm still with some movements) were offered and the snake accepted them. However, behavioral changes in subduing and swallowing prey were noticed. Snake caught the mouse and biting repeatedly while kept it grasped in their mouth. Continuous fast lateral head movements were recorded till the prey was still. Without release the mouse, the snake started ingestion moving maxillary and mandibular bones alternately. Swallowing time increased (17.00 ± 8.46) when compared with normal fanged specimens of the same size fed at same conditions (7.00 ± 2.13). The active movement of fangs during the swallowing has been documented, helping the snake to bring food into the esophagus. The fangless *B. moojeni* showed difficulty in swallowing, needing a greater number of jaw movements and consequently a longer time till complete the ingestion. However, digestion time up to fecal excretion did not vary among normal snakes (8.54 ± 3.62) and the fangless one (8.50 ± 5.24). The venom activity in poisonous snakes feeding is not yet clarified, and our results support that killing prey potentially dangerous by envenomation minimizes potential damages to the snakes, but not seems to affect significantly the digestion time. Predatory and feeding behavioral changes in fangless *B. moojeni* were proved by the fact that the snake does not strike and drop his prey already poisoned but catch the mouse and keep it grasped, swallowing only when its vital signs are absent. This is the first report of changes in predatory and feeding behavior in a viperid kept in captivity, which lost its fangs definitively but remains feeding spontaneously. On its natural habitat, the fangless snake possibly would take problems to subdue prey, endangering its survive.

8.02Squamata Reproduction Research Group: studies on reproduction of *Bothrops* snakes

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Introduction:*Bothrops* is the most diversified genus of pitvipers in the neotropical region: it is composed of more than 40 species that occur in Central and South America. Members of the Squamata Reproduction Research Group (SRRG) have been studying the reproductive biology of several species of lanceheads in an ecological and evolutionary approach. **Objectives:**The aim of this study is to divulge the research in reproductive biology of lanceheads that are being conducted by SRRG.**Methods:** A summary on the past and current research conducted by members of theSRRG was made to provide an overview of reproductive studies in this genus that are being conducted in the Laboratory of Ecology and Evolution at Instituto Butantan.**Results and Discussion:** Studies on reproductive biology of several species, including *Bothrops atrox*, *B. leucurus*, *B. moojeni*, *B. jararaca*, *B. insularis*, *B. neuwiedi*, *B. erythromelas*, *B. mattogrossensis*, *B. pauloensis*, *B. pubescens*, *B. diporus*, *B. jararacussu*, *B. alternatus* and *B. cotiara* have been conducted using individuals held in scientific collections and capture of individuals in the wild for *B. neuwiedi* from Minas Gerais. Microscopic analysis of the gonads and genital ducts of these species provide data on female reproductive strategies - including sperm storage and male reproductive cycles - processes of spermatogenesis in the testes, hypertrophy of the sexual segment of the kidney and male sperm storage in the ductus deferens. Field studies on the reproduction of *B. insularis*, an endangered species, have also been conducted since 2006, and these results are about to be published in an international journal. Reproductive tatics are really intriguing in *B. insularis* due to the intersexuality as all females analyzed so far have hemipenis. Studies comparing the reproductive strategies of different populations of *B. leucurus* and *B. jararaca* showed that some reproductive traits may vary intraspecifically. On the other hand, our results indicate that phylogenetic inertia also has an important role in the determination of the timing of reproductive cycles in the genus *Bothrops*. At this moment, students are carrying on researches on semen evaluation of captive *B. insularis* and *B. jararaca* and reproductive biology of several species of the genus, that intends to provide a comprehensive evaluation of the evolution of reproductive strategies in the genus, including the analysis of the influence of the actual climate conditions and phylogenetic relationships on the determination of reproductive timing in *Bothrops*.

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8.03Squamata Reproduction Research Group: Advances in reproductive biology of Neotropical rattlesnakes

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Introduction: The Squamata Reproduction Research Group (SRRG) was formed in 2010 at Instituto Butantan with the objective of producing scientific knowledge in the field of reproduction of snakes, lizards, and amphisbaenians, in a multidisciplinary way. This group is comprised by undergraduate and postgraduate students, trainee (Fundap), and pos doctors, some of which are involved with issues relating to the reproduction of rattlesnakes.**Objectives:** The aim of this work is to disseminate the results of research in reproductive biology of Brazilian rattlesnakes performed by SRRG. **Methods:** Results of the research conducted by SRRG using the Brazilian rattlesnake as a model, were compiled to present the scenario of the advances in reproductive biology questions to the genus *Crotalus*. **Results and Discussion:** Reproductive biology of *Crotalus durissus* from Northeastern Brazil: Size at maturation in both sexes and male reproductive cycles were markedly different from populations of Southeastern Brazil suggesting that these reproductive characteristics widely differ across the species'range. Reproduction costs in *Crotalus durissus* from Southeastern Brazil: Reproductive strategies adopted by females require a high energy investment - evidenced by the higher levels of abdominal fat and lipids in the liver during vitellogenic phase. Moreover, the tactics adopted by males during reproductive season (autumn), increase the rate of sighting and also is observed a higher intensity of secretory granules in the sexual segment of the kidney. Seasonal variation of body temperature in *Crotalus* maintened in Serpentarium: Pregnant rattlesnakes keep body temperatures significantly higher and less variable than males and non-reproductive females during the Summer, suggesting that higher temperatures are favorable for embryo development. Average body temperatures remain different during autumn in males and females. Corpus luteum: The influence of the corpus luteum in the maintenance and development of embryonic and gravidic structures was observed after fertilization. Sperm Storage: Female rattlesnakes have a obligatory pattern of sperm storage (UMT - Uterine Muscular Twisting) and to understand the mechanism of formation of this morphological change in the uterus (UMT), females have been artificially inseminated. All these data reveal that many questions on the reproductive biology of Neotropical rattlesnakes have been elucidated, promoting a expansion of understanding of reproductive strategies of snakes with some results that are already available in the international literature.

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8.04 Reproductive strategies of *Bothrops alternatus* (DUMERIL, BIBRON AND DUMERIL, 1854) from Minas Gerais, southeastern Brazil

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Introduction: The genus *Bothrops* (lato sensu) is a monophyletic group, with more than 40 species distributed from Mexico to Argentina. This genus can be grouped in six groups, among them the group alternatus, where the snake in study (*Bothrops alternatus*) is inserted. Few studies describe the reproductive strategies in snakes of the genus *Bothrops*. Occupying a basal position in the clade, the group alternatus has a big importance in studies of the biology and evolution of reproductive strategies. **Objectives:** The aim of this study intended to investigate many aspects of reproductive biology of populations of *B. alternatus*, from southeastern Brazil. **Methods:**We used specimens of scientific collections, where they removed fragments of gonads and genital tracts of males and females at different times of the year, in order to identify the main phases of the ovarian cycle, testicular and sperm storage in both sexes. **Results and Discussion:**The follicular development begins in the summer, copulations were recorded in autumn and in spring the ovulation occurs and then the fertilization. Thus, sperm storage becomes mandatory during winter. The results of macroscopic and microscopic analysis of the female *B. alternatus*' uterus, showed that the utero muscular twisting (UMT) is a stimulus found in this specie, with the presence of sperm storage in females. The births were documented in summer, so pregnancy lasts about four to five months, with an average of8.3 (± 6.4) offspring per female. In males, testicular development begins in late spring, with a peak of spermatogenesis in the summer, before copulation, indicating a cycle associated to males of*B. alternatus*. Therefore, the reproductivecycle is seasonal to both sexes. Preliminary data suggest that the cycle of this species can be conservative when compared to other populations of *B. alternatus* in colder regions, in other words, there may be no reproductive variability in different climatic conditions.

Supported by CNPq.

8.05Capture and maintenanceof*Bothropsatrox*inwestern Pará, Brazil

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Introduction: Studies on venoms from snakes collected in their natural habitats are important to understand ecological relationships and also the regional consequences in snake bites affecting human victims. However, such studies require keeping animals in captivity nearby their natural habitatfor obtaining most representative venom samples. **Objectives**: For further studies about the venom of *Bothrops atrox* from the western Pará, our initial goal was to organize a suitable environment and protocol to reduce mortality and minimize the stress caused by intensive captivity. **Methods**: Licenses for collection, transportation and maintenance of snakes in captivity were obtained (SISBIO 32098-3; CGEN 010269/2012-6). *B. atrox* was captured by pitfall traps with drift fence, occasional encounters and donation by third parties. The collects occurred from August 2012 to April 2013 in different environments (forest, savanna and pasture) including the municipalities of Santarém, Belterra and Oriximiná. The snakes have been kept in a room of about 17m² in a cycle of 12 hours light/dark at 25°C and relative humidity air around 70%, in Laboratório de Pesquisas Zoológicas, Faculdades Integradas do Tapajós, Santarém-PA. Endo-ectoparasites have been controlled by fenbendazole and metrifonate, infections by enrofloxacin 5% and topical nitrofural and terbinafine hydrochloride 1% was used as antifungal. Morphometric data have been recorded on individual sheets along with all information about the capture and handling in captivity. Venom was extracted soon after the capture of snakes and every 40 days following the initial extraction. Feeding occurred one week after each extraction. **Results and Discussion**: In total, 27 specimens were collected, 18 females and 9 males. And yet 73 individual venom samples were obtained from 21 juvenile and adult snakes. The amount of lyophilized venom was above of 1g in each extraction, sufficient to many studies about *B. atrox*. The organization of the herpetarium nearby the snake habitat improved the management of animals and allowed a good quality and quantity of venom production. This step will be essential for studying venom variability related to the ecology of snakes and its effects in snake bite accidents in Pará.

Supported by: CAPES

8.06Herpetofaunal surveys as tool to Payment for Environmental Services in Atlantic Rain Forest: a study case in Southeastern Brazil

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Introduction: The Payment for Environmental Services (PES) is a compensation program which yields economic incentive for owners of rural lands who supplant agriculture and pasture activities for conservation strategies. This mechanism of environmental policy can be an important tool to encourage the conservation of megadiverse ecosystems, such as Atlantic Rain Forest. Therefore, recognition and rating of the services provided by this ecosystem are the basis for calculating the financial support to owners. In this context, biodiversity data is crucial, and surveys of important faunal groups (e.g. herpetofauna) are relevant to compose the PES model. **Objectives:** The current study had the aim of surveying the herpetofauna at Lymington Foundation (LF), in Juquitiba, SP, providing data for the PES elaboration and modeling. **Methods:** This PES program was an initiative of Secretaria do Meio Ambiente and Fundação de Apoio à Universidade de São Paulo, supported by Fundação Boticário. The work was conducted at LF (-23.96478ºS -47.01452ºW), which has 36.30ha, of which 19.58ha possess native vegetation. Three surveys were conducted during October/2012, February and June/2013. The surveys consisted of nocturnal time-constrained search. Each survey lasted four days, and was conducted every day at a different track, comprising water bodies, forest, and pastures. The searches lasted 1:30h, totalizing 72 hours. Additional data were obtained by accidental encounters during the day, and also from scientific collections of Butantan Institute and Célio F.B. Haddad. **Results and Discussion:** 34 Anuran species were surveyed, belonging to eight families (three Brachycephalids, three Bufonids, one Centrolenid, one Craugastorid, 19 Hylids, two Leiuperids, four Leptodactylids, and one alien Ranid). Three species of lizards were found, two of Leiosauridae and one of Gymnophtalmidae. Only one amphisbaenian specie was found. Concerning snakes, 24 species were surveyed, three Colubrids, 18 Dipsadids, one Elapid and two Viperids. The highest amount of species was found in February. Based on species richness and composition, LF shows a good conservation status. Our study also highlights the importance of utilizing secondary data, avoiding an underestimation of species richness. Therefore, an adequate elaboration of PES should rely on primary and secondary data, with several campaigns, taking into account the best season for sampling the group of interest (i.e. rainy and warm season for herpetofauna). Reptiles and amphibians may be effective proxies to environmental services if appropriately sampled.

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8.07New methodologiesforadaptation ofCheloniakeptin the *Patio Casa VitalBrazil*(PCVB), Herpetology, InstitutoButantan.

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Introduction: Since 2008turtlesoriginating from theAnimal Reception are keptin asemi-extensive placeat thePCVB.Inimprovisedspaceandscarce resources, this site became apleasant and appropriate habitatwith multiplenichesfor the different allocated species.Currently44specimens are kept:*Trachemys*(20), *Geochelone*(16), *Apalone* (1), *Chelidra*(2), *Phrynops*(4), *Hydromedusa*(1): theylack provisionsand favorable conditionsforcaptive management and each specimen carry a microchip identification that allows the accompanimentthroughout their lives.**Objective**: To provideshelter accommodation, water, balanced diet, daily cleaning andveterinary procedures, requirements for thespecies welfare and development. To observebehaviors andminimize the lossof captive specimensthroughrequired interventions.**Methods:**In these fiveyears ofhandlingseveraladequacies were madeto the precincts stabilization and species adaptation. We have kept the tortoises grouped by species in PVC boxes and snapping turtles individually. Inside the pool are keptthe freshwater turtles, in glass terrarium thesoft-shell turtle, and loose in the courtyard, the tortoises. To preventloss duringthe colder months, shelters were adapted andheaterswere placed insidemostenclosures. Inside theenclosures we put sand, imitatingan artificial beach, and somespecimensthat have beenmatingsince 2010neededappropriate location forthe oviposition so they couldovopositin the groundorin the water.The implanted heatingforterrestrial speciesprecincts consistsof a plateof cementwithelectrical resistance,moisture and rain resistant.Inside the pool we have keptthe heater withthermostatandinside the terrariumswe usedaquarium heaters.**Results andDiscussions**:Theheating platesunder theshelter of fiber cement tiles provide abroadheatingon the surfacewith theincidence of sunlight, keeping the temperatureindecline at dawn.Theturtlescrowdsover themas the plates dissipateheat and,on different occasions, they dignestsin thesand bankplacedbetween the plates. Themicroenvironments insidePCVB havenatural photoperiodandweather, but not frequentlythe enclosurescoincidewith the species region occurrence, causingbehavioralstressandthermal discomfort.Thus, the specimens alreadyacclimatedare vulnerableto natural weather,making it necessary todevelop measures tominimizetemperature oscillations.Wehave implementedheatingand sandy beachesto offerbetter thermoregulation quality and generatewelfareto specimens. After these providences, there is a low rate mortality, especiallyin relation to diseasesrelated tolow temperaturessuch asPneumonia.

8.08Comparative study of bacterial adherence on skin fragments obtained from two amphibian species using an *in vitro* assay.

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Introduction: The amphibian skin is permeable and constantly moist, and therefore plays an important role in cutaneous respiration. As a result, these animals are dependent on humid environments, which favor the growth of various microorganisms on the skin. Some of these microorganisms can be potentially pathogenic and opportunistic, and are recently associated with population decline of amphibians worldwide, which is a major concern of ecology today. **Objectives:** This work aims the development of an *in vitro* experimental model to morphologically evaluate the characteristics of the interaction between various species of bacteria and amphibian skin, minimizing the number of individuals taken from nature for research purposes. **Methods:** Skin fragments collected from dorsal and ventral areas were collected from *Lithobates catesbeianus* (frog) and *Rhinella icterica* (toad), and maintained in culture medium. Fragments collected from each one of the species were individually infected by two samples of *Citrobacter freundii*, *Escherichia coli* and *Klebsiella pneumoniae*, and incubated for 24 to 48 hours at 37 °C. The infected fragments were then fixed and processed for histological and electron microscopy analysis. **Results and Discussion:** In general, the bacterial adherence to the skin of *R. icterica* was much more intense than the adherence observed in *L. catesbeianus*, regardless of the period of infection. The bacterial adherence was more intense on the ventral skin of *L. catesbeianus*, whereas in *R. icterica* was more intense on the dorsal skin. These results are possibly related to the skin characteristics of each specie(mainly texture and moisture content). Among the bacterial species studied, *C. freundii* and *E. coli* showed more intense adherence to the skin of both amphibians. In experiments using *R. icterica*, scanning electron microscopy analysis suggested that in longer periods of infection bacteria can penetrate the skin surface. The *in vitro* method presented here can be an efficient method to be used as a first approach in studies involving the interaction between bacteria and amphibians skin. This method also allows the performance of a large number of assays giving the advantage of saving the use of a much larger number of amphibian specimens which is usually required for *in vivo* studies. In this way, experiments using few amphibian specimens can be performed in order to *a priori* select, both amphibian and bacteria species which will be more useful for the conduction of further investigations.

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8.09Reproductive biology of *Bothrops pubescens* from Rio Grande do Sul, Brazil

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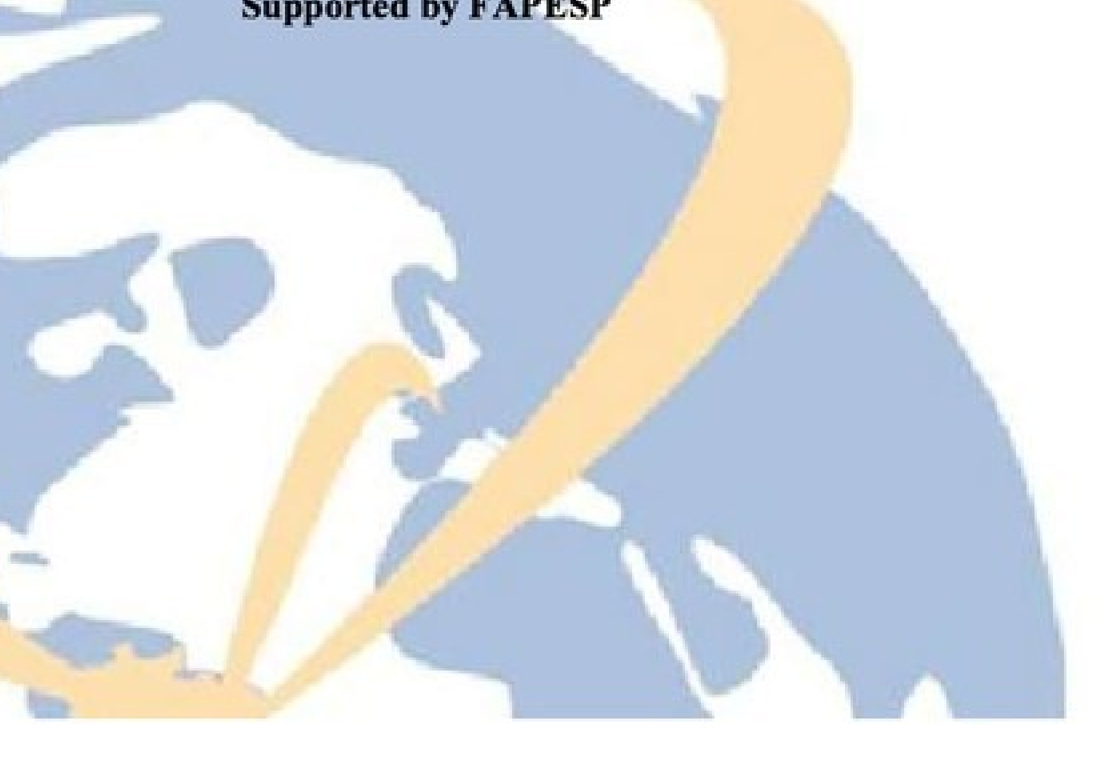
Introduction:*Bothrops pubescens* is a lancehead that belongs to the *B. neuwiedi* group. This snake is a common species in deciduous forests in southern Brazil. Previous studies on the reproductive biology of *B. pubescens* reported that vitellogenesis occurred throughout the year. Embryos were found in the oviducts from October to March and births occurred from January to March in captivity. There is a report of parturition in a *B. pubescens* female which was alone in captivity for some years. Authors hypothesized that fertilization occurred by stored sperm. **Objectives:** The aim of this study is to provide new data on male and female reproductive cycles of *B. pubescens* from Rio Grande do Sul in Brazil. **Methods:** We examined 70 individuals of *B. pubescens* (46 mature males, 19 mature females and 05 immature) held in the collection of "Pontifícia Universidade Católica" (PUC) of Rio Grande do Sul. Gonads, accessory sex organs and genital ducts of males and females were collected and processed to histological analysis. **Results and Discussion:** Testes length peaks in summer which suggests that spermatogenesis occurs during this season. It was confirmed by histological analysis of the testes: spermatogenesis occurred from January to July (summer, autumn and beginning of winter), synchronously to the period when court was observed in the wild (March). Individuals sampled in the same season show some degree of variability regarding the spermatogenic stage, characterizing a seasonal semisynchronous reproductive cycle. The sexual segment of kidney (SSK), an androgen-dependent organ, was active from January to July, synchronously to the spermatogenesis in the testes. Thus, male reproductive pattern may be characterized as associated. Results on female reproductive cycle confirmed the existence of a highly seasonal reproductive pattern, as observed in other *Bothrops* species. Early embryos (only yolk visible macroscopically) in the oviducts were observed in November and December. Uterine muscular twisting (UMT), which has already been described as a sperm storage structure in the posterior uterus of neotropical pitvipers, was observed in a female presenting secondary vitellogenic follicles in June. It reinforces the hypothesis of sperm storage in this species considering that ovulation is not synchronous to the observation of court in the wild (March) and mating in captivity (July). Anyway, histological analysis will be done to confirm the existence of sperm stored in the oviducts.

Supported by FAPESP



8.10Exploring new directions in reproductive biology of colubrid snakes

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Introduction: In the last two decades, our knowledge on the reproductive biology of Neotropical colubrid snakes has greatly advanced with the profusion of a number of studies. These studies have focused basically on the reproductive cycles (via macroscopic investigation of the gonads). This knowledge has provided an excellent background for the developing of new studies in different fields of reproductive biology of colubrid snakes. The Squamata Reproduction Research Group (SRRG) - comprised by undergraduate and postgraduate students, trainee (PAP) - has recently advanced in this direction. **Objectives:** Herein, we aimed to disseminate the results of recent research in different fields of reproductive biology of Brazilian colubrid performed by SRRG**,** such as nest-site selection, developmental plasticity, evolution of viviparity, sperm storage, and the role of sexual segment of the kidney. **Methods:** Results of the research conducted by SRRG using several species of Brazilian colubrids were compiled. These studies comprised field observations, egg incubation in different temperatures, dissection of eggs and preserved specimens, and histological examination of the male and female reproductive tract.**Results and Discussion:** Sperm storage in the reproductive tract is an important component of the reproductive cycle of both males and females. In addition, histological examination of a number of species have revealed several incongruences between macroscopic and microscopic analyses what reinforces the need to rely on histological evidence to correctly describe reproductive cycles (mainly male ones). In most snakes studied, the seasonal hypertrophy of the SSK is associated with mating period. In an ecological context, we found that colubrid snakes retain eggs in the uterus after ovulation and lay them with embryos between the end of organogenesis and early growth. Colubrid snakes use mostly anthills as nesting sites, but snakes also lay eggs within tree trunks, termite nests, and holes in the soil, among leaf litter, under stones, and on bromeliads. Nest-sites are used both singly and communally, but the type of use appears to be species-dependent. Nest-site selection by mothers may play a significant role in the reproductive success of snakes since our laboratory experiments showed that incubation temperature influence several phenotypic traits (such as developmental rates, hatching success, body size, body mass, and post-hatching behavior).

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8.11Evolution of viviparity in snakes of the tribe Hydropsini

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Introduction: Oviparity is the ancestral reproductive mode of reptiles, and viviparity evolved nearly 100 times in Squamata. The most accepted scenario for the evolution of viviparity suggests that it is an adaptation to cold climates and that it arises from progressive increases in the amount of intrauterine embryonic development before egg-laying. Moreover, evolution of viviparity requires the loss of eggshell to allow maternal-fetal gas exchanges. However, the timing and mechanism of eggshell loss are not fully understood. **Objectives:** We used water snakes (Hydropsini) as a model to test, within a phylogenetic framework, several hypothesis derived from the evolutionary scenario above. Specifically, we focused on three main questions: (1) Is the evolution of viviparity an irreversible phenomenon? (2) Is viviparity associated with changes in uterine morphology and eggshell thickness? (3) Is viviparity an adaptation to cold climates? **Methods:** Reproductive modes of 16 species of Hydropsini were characterized by the observation of reproductive output. Reproductive modes were then reconstructed on the phylogeny of the tribe. Histological sections of the eggs and uterus of females in different reproductive stages were performed to measure shell glands and eggshell thickness. Lastly, we characterized geographical distribution of Hydropsini species compiling records from zoological collections, what allowed us to draw the climatic profile of the occurrence area of each species. **Results and Discussion:** Hydropsini contains both oviparous and viviparous species. The evolutionary change to viviparity was accompanied by important uterine modifications, including the reduction of the glands that secrete the eggshell. The smaller glands may explain the absence of eggshell in viviparous taxa. We found no support for the hypothesis that acquisition of viviparity is irreversible since our analysis suggests that oviparity re-evolved in some taxa. Reversion to oviparity in Hydropsini may have occurred because the machinery responsible for eggshell secretion is not entirely lost in viviparous taxa. The hypothesis that the increases in the intrauterine embryonic development are accompanied by decreasing eggshell thickness was not corroborated. Lastly, we found no support for the widely accepted hypothesis that viviparity is adaptive to cold climates. Viviparity in Hydropsini may be a response to egg mortality due to flooding. Hydropsini nests in early rainy season in the riverbanks while the level of rivers is rising. If eggs require a long incubation period nests could be flooded before hatchings. By retaining eggs for longer periods, females oviposit with embryos at late stages, therefore decreasing remaining incubation period allowing eggs to hatch before the river levels elevate and flood nests.

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8.12Feeding behavior of *Tomodon dorsatus* in captivity

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Introduction: Feeding habits of snakes are an important aspect of their ecology and evolutionary history. Snakes that feed on slugs (goo-eaters) are ecologically diversified, and represent an excellent model for the study of feeding specializations in snakes. **Objectives:** The objective of the present work was to describe the feeding behavior and mechanism of *Tomodon dorsatus* in captivity. **Methods:** Two adult males and females of *T. dorsatus* kept in captivity at the Museu Biológico of Instituto Butantan were studied for that purpose. The snakes were kept in polypropylene boxes (34 x 22 x 12 cm) lined with *Sphagnum* sp., with shelters and water *ad libitum.* In order to observe the feeding behavior, slugs of the family Veronicellidae were offered every 15 days. Feeding behavior sequences, ingestion time, and the number of lower jaw movements during ingestion were recorded in films at 60fps using a PANASONIC HDC-900 digital camera. Images were analyzed in order to establish the feeding mechanism as well as details of the feeding behavior exhibited by this species. **Results and Discussion:** Prey ingestion time and the number of lower jaw movements used by *T. dorsatus* is similar to other "goo eater" snakes. The feeding behavior of the species comprised active search, inspection, capture, handling, ingestion, and post-ingestion (after swallowing). Searching behavior was resumed after post-ingestion, indicating that this snake can feed on more than one slug per foraging period. Prey elevation from the ground was observed in *Tomodon* as well as in other slug eating snakes, and may help reduce the incidence of solid particles adhering to the body of the slug and facilitate handling of viscous prey. The feeding sequence observed in *T. dorsatus* is similar to that observed in other "goo eater" snakes of the genera *Dipsas*, *Sibynomorphus*, and *Sibon*. Although *Tomodon dorsatus* lacks the morphological specializations known to be present in the dipsadine "goo eaters", its efficiency in handling and ingesting its preys seems to equal the one shown by species from the latter group, suggesting that *Tomodon* encountered a distinct and still enigmatic solution to handle viscous preys.

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8.13 Thermal influence on quantitative measurement of residual yolk in neonates of *Bothrops jararaca*.

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Introduction:The environmental temperature is an important factor for the homeostasis of ectothermic animals such as snakes. In their first weeks of life these animals present in their coelom, near the intestine, certain quantity of residual yolk, important for the nourishment of the neonate until its first meal. This substance wanes as long as it is absorbed. Therefore it is expected that the temperature influence the rate of absorption of this energetic resource. **Objectives**:This experiment aims to gauge the effects of the room temperature on the pattern of absorption of the residual yolk in two newborn *Bothrops jararaca* groups, maintained at different temperatures. **Methods**: To this experiment three clutches of *B. jararaca* were selected as soon as they were born, having weight, snout-vent length and total length measured on the date of birth. The animals of the experimental group were kept at an average temperature of 25ºC in an incubator with an internal photoperiod of 12 hours triggered by fluorescent lamp. On the other hand the animals from the control group were maintained at room temperature ranging from 20ºC to 25ºC and kept in the triage room at the Laboratório de Herpetologia. All the animals were maintained in plastic cages with corrugated substrate and water at their disposal, but they were not fed. In the experimental group, the maximal and minimal incubator temperature was measured daily using a digital thermometer. In the control group the maximal and minimal temperature inside and outside the cage were daily collected. Once per week, one male and female from each group were submitted to euthanasia in saturated CO2 chamber, within a few days of interval, alternating the control group and the experimental group. Each animal was weighed, necropsied and had the liver, abdominal fat and residual yolk weighed on an analytical balance. The data collected were subjected to least squares statistical test. **Results and Discussion:** The results obtained by the method of least squares support the hypothesis of the influence of temperature on the absorption of residual yolk, demonstrating that the average speed of consumption of the residual yolk by the experimental group was approximately 7.8 times higher when compared to the speed of consumption of the control group, and the average daily consumption for the experimental group was approximately 0.03g/day while in the control group the average daily consumption was approximately 0.004g/day.

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8.14Analysis of *Bothrops jararaca*peripheral blood leukocyte populationusing flow cytometry

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Introduction: A critical function of the cellular inflammatory response is the recruitment of leukocytes to the lesion and their activation to perform host defense. Leukocytes phagocyte aggressors, destroy bacteria and other microorganisms, and eliminate necrotic tissue and foreign substances. Due to the great variation of snake's leukocytes on cytochemistry, an analysis focused only on morphology is not sufficient to determine the different cell types. Thus, additional study of immunohistochemistry, histoenzimology, flow cytometry, among others, are important to better understand the function and origin of each cell type. **Objectives**: Characterize specific cell types and subtypes according to membrane characteristics, size, presence of granules and internal complexity (organelle) by flow cytometry, enabling the qualitative and semi-quantitative assessment of cells constituting the immune system of *B. jararaca*. **Methods**: Blood samples were collected by caudal venipuncture in tubescontaining sodium heparin from ten *Bothrops jararaca* (five males and five females) from the Laboratório de Herpetologia. Fresh blood was centrifuged in the presence of either two commercial gradient for leukocytes separation: Ficoll-Paque PLUS® and Percoll®. After centrifugation, leukocytes were analyzed according to size and internal complexity by flow cytometry in FACScalibur (BD Biosciences). **Results and Discussion**: There were no difference in the distribution of leukocyte population between specimens. Four distinct leukocyte populations were verified: heterophils, small lymphocytes, large lymphocytes and azurofils. In this study we could not characterize basophil population. Although there were no statistical differences betweencell populations isolated by the two different gradients, leukocyte morphology was better preserved with the use of Ficoll-Paque PLUS®. Our study standardized the leukocyte cytogram in *B. jararaca* and identified the composition of the obtained populations.

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8.15Morphology of the poison glands in the toad *Rhinella granulosa* during ontogenetic development

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Introduction: The ontogenetic development of the amphibian integument, particularly their skin glands during the process of metamorphosis, is very little known. These glands, which constitutes a synapomorphy of the three orders of the living amphibians, undergo a process of differentiation in order to develop poison glands and mucous glands, used in defense against predators and microorganisms, and against desiccation. **Objectives:** We aim at the study of the differentiation of the skin (particularly of the skin glands) of the toad *Rhinella granulosa* in different larval stages and after metamorphosis. **Methods:** We collected skin samples from various larval stages of *R. granulosa*, from the semi-arid region (Caatinga), following the classification of Gosner (1960). Tadpoles and skin fragments of juveniles were fixed in Karnowsky, and embedded in paraffin or historesin for light microscopy, and epoxy resin for transmission electron microscopy. Animals, either entire or sagittally sectioned, and skin fragments were also prepared for scanning electron microscopy. The histological samples were stained with hematoxylin-eosin (paraffin sections) or toluidine blue-fuchsin (historesin sections). The von Kossa reaction was also performed for detection of a calcified dermal layer within the skin. For ultrastructural analysis ultrathin sections were examined under a transmission electron microscope. **Results and Discussion:** The skin glands begin to develop from Gosner's stage 36, in which the epidermal cells proliferate and invade the dermis.From stage 41 venom glands spread throughout the skin, similarly to what is observed in adults. These glands are syncytial and are involved by a distinct unicellular layer of myoepithelial cells. In stage 43, in which metamorphosis is still incomplete, the venom glands of the region equivalent to the adult parotoids (next to the timpani) appear larger and more numerous. Just after metamorphosis is complete, the toadlets show larger glands in this region, but their secretory content still resembles the regular skin glands of adults. The cutaneous changes observed in the tadpoles and toadlets are associated with their gradual adaptation to their adult life in the terrestrial environment, preparing the animals for terrestrial chemical defense against predators and microorganisms, typical of all amphibians.

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8.16Osteitis deformans in snake *Bothrops leucurus*. Use of an acupuncture technique (moxibustion) as a supplementary therapy

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Introduction: Osteitis deformans, also known as Paget's disease (in humans), is a disease that causes deformation in bone remodeling, with bones appearing as a mosaic of cement or reverse lines which are bone resorption and deposition surfaces. For reptiles, this term has been widely employed to describe several vertebral lesions, and particularly in snakes the presence of ankylosis is very common. The causes of the disease are not well defined, but autoimmune or viral infections are some of the possibilities. Acupuncture is a method widely used for orthopedic diseases, and can be used to vasodilation, anti-inflammatory, analgesic and healing. One specimen of *Bothrops leucurus* from the collection of Museu Biológico of Instituto Butantan, with nine years old and born in the same Institution, was found with no movements and with the thoracic region of the spine swollen. Diagnostic through palpation has shown the presence of local edema, pain and absence of fractures. **Objectives:** To describe the use of acupuncture (moxibustion) as an auxiliary method in the treatment of osteitis deformans in a snake. **Methods:** Radiological examination was performed in the Veterinary Hospital of the University of São Paulo, showing bone proliferation and ankylose ventrolateral to the vertebral bodies and facet joints of the vertebral bodies of the spinal segment mediocranial, consistent with the framework of osteitis deformans. The specimen was initially treated with anti-inflammatory and analgesic ointments, and subsequently with moxibustion once a week for 5 to 10 minutes, during three months. **Results and Discussion:** After the conventional treatment, the animal showed decreased edema and improved posture. After three months of treatment with moxibustion, the animal was clinically evaluated and showed a better posture, locomotory performance, feeding and defecation compared to the condition in the beginning of treatment.Moxibustion technique could be easily performed on the animal without stress or the need of contention. Although the results are not conclusive and more experiments are necessary to prove the benefits of the technique, these findings can suggest that moxibustion might be a good complementary therapy to the traditional veterinary treatments.

8.17Who is the mother? Sonographic evaluation of the reproductive postpartum *Eunectes murinus* (Anaconda)

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Introduction: *Eunectes murinus* is a semi-aquatic Neotropical snake that inhabits the large hydrographic basins of the tropical South America. Considered the second largest snake in the world, some specimens might reach 10 meters in length. Anacondas are viviparous snakes, with the foetuses completing its development inside the oviduct (uterus) during the six-month pregnancy period. Physical examination is limited when dealing with reptiles, and the semiology process of internal organs is difficult in this species due to its body size. Ultrasonography is a viable solution for the study of internal organs, especially those of the reproductive system, causing minor discomfort, as it is a painless and non-invasive method of medical examination. The enclosure of *Eunectes murinus*at the Museu Biológico, Instituto Butantan houses four snakes: two adult females, one young female and one adult male. In July 2013, one litter with 17 young was born, and any of the two females (MIB4115 and MIB4289) could be the mother. **Objectives:** The objective of this research was to examine the two anaconda females in order to determine which one was the mother of the litter. **Methods:** Twelve days after birth of the litter, both females of *Eunectes murinus,*measuring approximately four meters each were ultrasonographically evaluated. Both snakes were physically contained with a herpetological hook, and then manually for approximately 15 minutes. Evaluation was consisted in applying acoustic gel over the snakes's skin, and positioning the transducer ventral-laterally in both left and right sides. **Results and Discussion:** The ultrasonographic exam on the specimen MIB4115 showed ovarian follicles located caudally relative to the gallbladder, with elongated shape, heterogeneous and hyperechoic, disposed in a line and ranging from 1.03 to 3.14cm diameter. These characteristics indicated that follicles in this specimen were in the vitelogenic phase. Specimen MIB4389 presented an absence of ovarian follicles, with a thickened and irregular oviduct (uterus) measuring 5cm length, with a hyperechogenic image. The absence of follicles together with the thickening of the oviduct indicated that MIB4389 gave birth to the litter.

8.18Topographical anatomy of the bushmaster (Serpentes, Viperidae)

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Introduction: The bushmaster (*Lachesis muta*), Brazil's largest venomous snake, belongs to the family Viperidae and can reach 3.5 m in length and weigh around 6 kg. This species has crepuscular habit and is distributed in tropical forests withhuman low population density. *L. muta* is an oviparous snake and thefemales brood their eggs until hatching. In Brazil,the species is distributed in the states of Acre, Amazonas, Amapá, Pará, Rondônia, Roraima Alagoas, Pernambuco, Sergipe, Paraíba, Ceará, Rio Grande do Norte, Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro. Until 2004 two subspecieswere recognized: *L. m. muta* (Amazon basin) and *L. m. rhombeata* (atlantic rainforest - northern of Rio de Janeiro to Paraíba). However, it was seen that it is a monotypic species, with only one species,*Lachesis muta,* in Brazil. Due to deforestation, the genera is in rapid decline, having been included in the Red List of Threatened Species "International Union for Conservation of Nature and Natural Resources' (IUCN, 2000). In 17 years (1994-2011) the Instituto Butantan received only 10 specimens. **Objectives**: The knowledge of the topographic anatomy of a specific species, besides being a key to understand the functioning of the organism and its pathologies, is essential in the clinical examination and interpretation of diagnostic images. **Methods:** Since the distribution of organs in snakes of the same species has a constant relationship with snout-vent length (observed in *Crotalus durissus, Micrurus corallinus* and *Bothrops jararaca*), this study used two adult snakes (one male and one female) from Northeast of Brazil, that died from natural causes. The animals were dissected and their organs examined for its position in centimeters (from the snout) and the percentage in relation to their snout-vent length (SVL). **Results and Discussion**: No differences were observed among the position of the organs in male and female specimens. The bushmaster is the only Brazilian Viperidaethat has no tracheal-lung. The knowledge of this particularity is critical to the maintainers, as a simple deworming can lead the animal to death by suffocation, if it is parasitized by pentastomid (lung parasites) that reach 14cm in length and 5mm in diameter. The trachea of *L.muta*, located 24% of the head in relation to its SVLis 20% smaller than the tracheal-lung of *Bothrops jararaca* and *Crotalus durissus*, and its heart and liver are 10 to 20% more cranial respectively. All organs of *L. muta*are positioned quitecranially compared to the positioning of the same organs in *B. jararaca* and *C. durissus*. *Bushmasters* can have an average of 230 ventral scales, being more practical and faster locating their organs by percentage termsthan by counting the ventral scales.

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8.19Growth Differences Between Males and Females of *Bothrops jararaca*.

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Introduction:*Bothrops jararaca* is found in the south of Bahia, in the Southeast region, in the states of Paraná and Santa Catarina, reaching Mato Grosso and Goiás states. It occupies a wide range of habitats, from rainforests to open fields. While the females can reach up to 160cm, the males are smaller reaching 80 cm. An animal's body size may determine the animal's food habits, its vulnerability to predation, and its reproductive output. Clearly, growth rates are very flexible, and are influenced by intrinsic factors as well as by local resource availability. It is known that young *B. jararaca* feeds on frogs and small lizards and, as adults,on birds and small mammals. As males tend to be smaller than females, they feed upon ecthotermic preys for longer periods. When does the length and weight of females outdo those of the males? **Objective:**Determine the difference in growth between males and females of *Bothrops jararaca* born in captivity.**Methods:**Thirty two *Bothrops jararaca* (9 males and 23 females) born in captivity in 2011were used in this study.The snakes were divided into two groups: control group (6 males and 14 females) and experimental group (3 males and 9 females). The animals of the control group were fed every 15 days during the first year and every 30 daysin the second year with 10% of their body weight in live mice, while the animals of the experimental group wereforce-fed with exactly the same weight ratio. Both groups were measured routinely.**Results and Discussion:**No statistical differences were seen in weight or snout-vent length between sexes in the first two years of the snakes in either group. As *B. jararaca* usually attain sexual maturity at 3 years of age, differences of growth may then be present, when sexual hormones are in full activity. In this way, this work will continue for a couple of years or more to determine when the females of this species become bigger than the males.

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8.20Microevolution in the malaria vectors *Anopheles cruzii* and *An. homunculus* in two climatic seasons.

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Introduction: Females of *Anopheles cruzii* and *Anopheles homunculus* are morphologically similar and both occur in sympatry in southeastern Brazil. In this region, these species are considered respectively the primary and the secondary vectors of *Plasmodium spp.* Some biological processes in mosquitoes such as pathogen transmission are possibly affected by variations across climatic seasons. Despite the epidemiological importance of *An. cruzii* and *An. homunculus*, there have been few studies on the temporal dynamics of these vectors. **Objectives:** The aim of this study was to characterize populations of *An. cruzii* and *An. homunculus* withregard to genetic and morphological polymorphism in two different seasons: summer and winter. **Methods:** All specimens were collected in the Atlantic Forest in July 2011 (winter) and January 2012 (summer). We used wing shape as the morphological marker, according to standard geometric morphometrics methods. A 400-bp 3′ end of the mitochondrial gene CO-I was sequenced and used as the genetic marker. **Results and Discussion:** In both species, individuals clustered into two distinct groups in the morpho-axis of canonical variates according to season. Pairwise cross-validated reclassification showed that wing shape changed significantly during the time interval examined. Genetic analysis revealed rich haplotypic diversity (0.97) and high nucleotide diversity (0.012) within populations of *An. cruzii*. On the other hand, *An. homunculus* exhibited a slighlty lower haplotypic diversity (0.84) and moderate values of genetic divergence between seasons ($\square_{st} = 0.116$), suggesting that summer and winter populations are partly different. Despite being morphologically and phylogenetically very close, the species *An. cruzii* and *An. homunculus* have distinct genetic patterns, where *An. homunculus* does not have a haplotypic patrimony as rich as its congener. The interval between winter and summer is enough for species to develop both morphological and genetic variation. These changes appear to be rapid in these species and should be taken into consideration when developing vector control strategies.

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8.21Temporal variation in *Aedes aegypti*

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Introduction: *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) is the most important vector of dengue viruses in Brazil. As the vaccine against dengue is still under development, the only way of preventing the disease is to control the vector. Currently, the control methods have variable efficiencies and microevolution of mosquitoes is a limiting factor. **Objectives:** This work aimed to detect and estimate microevolution of *Ae. aegypti* in Butantã neighborhood (São Paulo city) during one year. **Methods:** Larvae of *Ae. aegypti* were collected monthly between March 2011 and May 2012. The five samples were pooled according to climatic seasons: fall/2011, winter/2011, spring/2011, summer/2011-12 and fall/2012. Right wings of 150 females (30 per climatic sample) were mounted in a slide-coverslip, photographed and had 18 landmarks digitised. Landmarks consisted of conspicuous and homologous wing vein crosses and its Cartesian coordinates were submitted to geometric morphometrics standard analyses. To assess the genetic variability, DNA of individuals were extracted and amplified for the six microsatellite *loci*: AED19, C2A8, T3A7, A10, B07 e B19. Evolution of morphologic and genetic characters during the studied period was evaluated by comparisons between seasonal samples. **Results and Discussion:** All *loci* were polymorphic and exhibited allelic variation across the seasons. Despite the polymorphism, overall genetic differentiation was low ($F_{st} = 0.0422$). Pairwise genetic differentiation between the five seasonal samples were low but significant ($0.03 \leq F_{st} \leq 0.05$; $p < 0.05$). Wing shape varied across the five seasons ($Q_{st} = 0.4732$). Discriminant analysis permitted highly accurate season identification based on wing shape (cross-validated scores ranged from 60% - 83.3%). Furthermore, pairwise Mahalanobis distance between seasonal samples also indicated significant wing differentiation ($1.99 \leq MD \leq 4.05$; $p < 0.0001$). We conclude that *Ae. aegypti* suffered microevolutionary processes in a period as short as one year. Both morphological and genetic markers agreed in detecting microevolution of *Ae. aegypti* during the studies period. In spite of the concordance between the biological markers, the speed of morphological variation was greater than genetic variation ($Q_{st}>F_{st}$), suggesting an action of natural selection on wing shape.

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8.22The parotoid macroglands of toads as a defensive arsenal: a morpho-functional view

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Introduction: Amphibian defense from predators and microorganisms is mainly carried out through skin glands that produce toxinsknown as "poison glands" or "granular glands." Among the anurans (toads, tree-frogs and frogs) such glands show a peculiar morphology that differs from that found in other terrestrial vertebrates. Anuran poison glands are devoid of a lumen and formed by a syncytium characterized by a non-walled multinucleated mass of cytoplasm, within which a large number of granules are produced and stored. In some groups such glands accumulate in certain regions of the body, forming prominent multiglandular structures (or macroglands), such as the parotoids of toads.In this case, when an external force is exerted onto a parotoid by, for example, a bite, the poison is expelled from the interior of the syncytial secretory units in the form of jets, directly inside the predator's mouth. This process characterizes passive defense, typical of amphibians. **Objectives:** To evaluate the adaptive advantages of the macroglands as multiglandular, syncytial structures in the passive chemical defense system of toads. **Methods:** The parotoids of toads from the neotropical species *Rhinella icterica* (from the Atlantic Forest, n=8) and *Rhinella marina* (from the Amazonian Rainforest, n=8) were manually compressed in their middle portion. They were then sectioned horizontally along the longer axis for internal examination. Fragments of the secretory units of the compressed region were fixed and processed for histology and electron microscopy. The average number of parotoid secretory units was defined by direct counting and compared between the two species by Student's t-test.**Results and Discussion:** In both species, most sectioned secretory units revealed to be intact and full of poison, except those located in the medial compressed region. Ultrastructurally, the syncytium showed to be composed of two distinct areas, one peripheral secretory area, usually highly electron dense, and a central area where the electron lucent poison granules of different sizes are stored, immersed in a cytoplasmic mass. The *de novo*synthesis of these granules gradually refills the glandular units inside out, compressing the surrounding tissue and increasing the internal pressure exerted by the poison. Thus, the syncytial organization of the units may favor the rapid replacement of the poison, without the barriers imposed by cellular membranes. On the other hand, the multiglandular organization of the parotoid may benefit from the dynamics of passive defense, maintaining the chemical "artillery" ready to operate in case the toad is reached by new bites.

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8.23The skin secretion of the tree frog *Corythomantis greeningi* from the Brazilian Caatinga: a biochemical and toxinological study.

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Introduction: Amphibian skin glands produce a surprising number of different molecules, many with a variety of biological actions used in chemical defense against predators and microorganisms. The Brazilian tree frog *Corythomantis greening* (*Cg*), endemic of the Brazilian Caatinga, produces an abundant skin secretion, which still remains totally unknown. During collecting of specimens in the field, this secretion showed to cause severe pain when it accidentally penetrates through small injuries in the skin of the collectors. Based on this fact, we aimed to characterize the skin secretion of *Cg* and study its toxic and nociceptive activities, correlating this data with the chemical defense of this species and with the possible prospection of bioactive compounds. **Methods**: The secretion was analyzed by SDS-PAGE and LC-MS, using a C18 column in positive ionization mode. The nociceptive and edematogenic activity was accessed using doses of 30, 15, 3.75 and 0.9 µg in right hind paw of Swiss mice (n = 6) and PBS was used as negative control. In the present work, we also investigated the cytotoxicity of the secretion on murine melanoma cells (B16-F10) and on murine fibroblasts cells (L929). **Results andDiscussion:***Cg* skin secretion has proteins distributed throughout the gel, with more abundance between 30 and 60 kDa. Molecules of small masses (200 to 500 Da), typical of steroids and alkaloids, are also abundant. The tree frog skin secretion induced nociception and edema in mice at all doses tested. Edema remained up to 96 hours in the higher doses Cells were incubated with increasing concentrations of *Cg* secretion (ranging from 0.78 µg/mL to 400 µg/mL). After 48 hours, the cell viability was determined using an MTT based assay. Our results indicate that the *Cg* secretion is highly toxic to the B16-F10 cells, inducing cell death even at 6.25 µg/mL concentration, while the fibroblast were only affected with the higher concentrations. The skin secretion of *Cg* is complex and comprises a number of proteins together with many other smaller molecules, probably steroids and alkaloids. More detailed experiments are necessary in order to better characterize all these molecules. The secretion showed considerable nociceptive activity at all doses tested. It also showed cytotoxicity in both used cell lineages, but the activity was more intense in B16F10, indicating a possible antitumor effect.

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8.24Reproductive and behavioral aspects of two sympatric lizards (*Enyalius*) of the Atlantic forest

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Introduction: The lizard species*Enyalius iheringii* and *E. perditus* are endemic to the Brazilian Atlantic forest and are sympatric in some areas of southeastern Brazil. Little information is known on biological, ecological, and mainly reproductive aspects of these two species.**Objectives:** Herein, we provide original information on reproductive (courtship, egg-laying, hatching) and behavioral (thanatosis) aspects of these two species from São Paulo state, southeastern Brazil.**Methods:** Field observationof *E. perditus*was taken during a herpetofaunal survey in a fragment of preserved Atlantic Forest in the municipality of Juquitiba, State of São Paulo, Brazil,and collection of specimen of *E. iheringii* was carried outin the municipality of Ubatuba, São Paulo State, Brazil.**Results and Discussion:**One male and one female (in shedding process) of *Enyaliusperditus*were found together in copulation attempt in mid-spring. Both were on a tree trunk about one meter above the ground. The male individual bit the female's neck and then both fell from the trunk on the leaf litter. After the fall, the male remained on the back of the female biting her neck in an attempt to immobilize it.Both remained in this position about 30 seconds. After this period, the female disengaged from the male and escaped through vegetation.Our observation was the first courtship confirmed taken in field conditions for a species of *Enyalius*.The pattern of courtship behavior observed here in nature was similar to that published for *E. perditus* was made in captive conditions and the shedding process is associated with the mating season of lizards and snakes, suggested that shedding is part of the estrus signal. Inlate summer, a gravid female of *E. iheringii* was collected and by early autumn it laid 18 eggs. Eggs averaged 15.0 ± 0.5 mm in length, 9.7 ± 0.7 mm in width, and 0.81 ± 0.10 g in mass.Thirteen eggs were incubated. Eight eggs spoiled over incubation period. Hatchingsoccurred in mid-winter, after an incubation period of 143 days. Hatchlings averaged 27.5 ± 3.5 mm SVL, 49.0 ± 8.5 mm in tail length and weighed 0.77 ± 0.01 g. This is the first report of egg-laying, incubation period, and hatchling size of a *Enyalius* species.Gravid females of *E.iheringii* were previously found in December/January and February suggesting that species presents some reproductive seasonality. Our observation of egg-laying in *E.iheringii* in April corroborotes this assumption but suggests that oviposition period in the species may extend further (to early autumn) than previously thought.

8.25*Phoneutria nigriventer*: feeding and venom production

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Introduction:*Phoneutria nigriventer* (Keyserling, 1891)is considered aspider of importance to public health in Brazil, because its venom has effects in human beings. The envenomation can cause pain, edema, tachycardia and possibly death. For the Minister of Health in 2012 there were 3,575 accidents registered in Brazil. At the Hospital Vital Brazil there were 62 registered cases in the same year. These spiders are called armed spiders because when they are in danger they raise theirs anterior appendages off substratum, elevating the cephalothorax. The venom used in antiarachnidic serum (SAA) is obtained at Laboratório de Artropódes of Butantan Institute. To increase the production of venom, we have changed its feeding. **Objectives:** Evaluate the influence of the feeding of *P.* nigriventer in the obtainment of venom. **Methods:** For thirteen months (July 2012 to July 2013), the venom produced was evaluated and the spider mortality observed. We studied two groups, one of them received food twice a month (GA) while the other group received food once a month (GB). The prey offered was cockroach (*Naphoeta cinerea*). **Results and Discussion:** Thevenom obtained was the same in both groups (Mann-Whitney, p=0.8777) but the group A, that received cockroach twice a month, produced during this period 521.0 mg, while the group B produced 497.7 mg. Also it was not observed difference about the mortality. Females have longer life in captivity than the males. Apparently, females died after the second extraction of venom (30%) while the males died after the first (35%). Females produced more venom than males, 2.23 and 0.55 mg/individually, respectively. Despite there was no difference observed between the groups studied, we obtained 23.34 mg more from the first group and it was very important for the Laboratory. Furthermore, when the spider has a chance to eat twice, we observed that the mortality is lower, because 71.8% of them died when they ate once. This work shows us how important it is to study methods to maintain spiders in captive conditions. When we compare July of 2012 to July of 2013, the venom quantity obtained in 2013 was 512.8 mg higher.

8.26Multiple paternity and parthenogenesis in the reproductive strategies of the Neotropical pitvipers *Bothrops* species (Viperidae, Crotalinae) based on microsatellite analysis

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Introduction: Reproductive cycle of neotropical pitvipers genus *Bothrops*(sensu lato) has been described as quite similar to that of species from temperate hemisphere, concerning female sperm storage, gestation and episodes of parthenogenesis. However, there are few genetic evidences which testify such findings mainly due to lack of molecular tools. Microsatellites are quite strong markers to address these questions, but they are scarce in literature for pitvipers. **Objectives:** In this work we analysed eight litters of *Bothrops* born in the Laboratório de Herpetologia-Instituto Butantan, using primers originally developed for *Bothrops insularis* and successfully transferred to four species of *B. atrox* species complex in order to check for the presence of polyandry and parthenogenesis events. **Methods:** Pregnant females of *B. moojeni* (n=3) and *B. marajoensis* (n=1) caught in wild had their offspring (n=34) investigated for seven loci of microsatellites assigned as: 52.17; 52.22; 52.7, 52.8, 60.3, 60.6 and 60.9. The same procedure was carried out with newborn (n=6) and their captive virgin mothers of *B. atrox* (n=1) and *B. moojeni* (n=3). Total DNA was obtained from liver tissue kept in 80% ethanol and blood samples collected in EDTA and kept in SDS/Tris solution using DNeasy Blood & Tissue - Kiagen. Excepting for primers 52.7 and 60.9, the remaining five loci tested produced amplification product in taxa investigated after PCR optimization. **Results and Discussion**:Polymorphism in different levels was observed among primers in all species examined. Number of genotypes varied from 21 (loci 52.17 and 52.22), 11 (52.8), 5 (60.3) and 2 (60.6) in *B. atrox*; 11 (52.17), 8 (52.22), 4 (52.8), 1 (60.3) and 3 (60.6) in *B. marajoensis*; and 31 (52.17), 24 (52.22), 11 (52.8), 1 (60.3) and 2 (60.6) in *B. moojeni*. Newborn and their virgin mothers presented identical genotypes for each of the five tested loci both in *B. atrox* and *B. moojeni*. In one same litter of wild *B. moojeni* from the State of São Paulo, born in captivity, it was possible to observe the presence of five different alleles for locus 52.22, indicating the genetic contribution of more than one father to the same offspring. Results presented here corroborate the existence of parthenogenesis as a more common than expected component of pitviper reproduction and occurrence of polyandry in the genus *Bothrops*.

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8.27Sexual behaviour of *Loxosceles amazonica* Gertch 1967 and *Loxosceles variegata* Simon, 1897 (Araneae, Sicariidae).

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Introduction: In *Loxosceles* Heineken & Lowe, 1832, Sicariidae family, there are 103 known species and 13 of these species occur in Brazil. These spiders are popularly called brown spider and are responsible for 38 % of the spider related accidents every year in Brazil. The knowledge about the reproductive biology or any other biological spect involving species *Loxosceles* is extremely important, considering they are synanthropic spiders and important to public health. **Objectives:** The aim of this study was to observe and describe all attitudes and steps of the sexual behavior of *L. amazonica* and *L. variegata*. **Methods**: In laboratory conditions, 53 pairings were formed randomly: 23 couples of *L. amazonica* and 30 couples of *L. variegata*. The attitude shown by pairs, during the steps involved in breeding, were identified and recorded with a camcorder Picasa model, to compare the behavior of the species. The spiders were placed individually in plastic containers of 65 mm in diameter. Each spider received weekly a nymph of *Phoetalia pallida* (cockroaches) or a nymph of *Grillus* sp. (cricket). The experiments were conducted in "arena" (box) transparent acrylic (500 x 200 x 200 mm), having three removable covers, two front and one center. **Results and Discussion**: Of the nine mating were obtained from *L. amazonica* and 10 from *L. variegata*. There described and recorded 20 behavioral attitudes along the five stages: pre-courtship, courtship, pre-copulation, copulation and post-copulation. The average time spent by *L. amazonica* and *L. variegata* in each step, respectively, was: in pre-courtship 10.8 and 202 seconds, in courtship 11 and 405.5 seconds, in pre-copulation 6.5 and 1770 seconds, in copulation 13.5 and 56.5 seconds and in post-copulation 1 and 828 seconds. The behavioral attitudes observed between the two species showed specific patterns in the sequences displayed by couples of each species. Changes in behavior exhibited in each step are related to a sequences of attitudes, number of copulations obtained and time spent among species.

8.28Inventory of the small mammals of a small fragment of the Atlantic Coastal Forest in Itanhaém, São Paulo, Brazil.

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Introduction: Originally, the Atlantic Forest covered part of 17 Brazilian States, presenting different landscape features as well as significant changes in the vegetation and climate with altitude and latitude. Together with the diversity of phytophysiognomies, the Atlantic Forest harbors one of the richest fauna, and a large number of endemic species. It is among themost devastated and threatened biomes in the world, due to the exploitation that began during the colonization of Brazil and is still a problem. The scientific knowledge about the species that inhabit the 5% remaining from the original Atlantic Forest is still incomplete, and new species and even genera of mammals are still being described. Small mammals play an important ecological role, and various studies indicate that marsupials and small rodents exert an important influence on the dynamics of Neotropical forests, being also good indicators of local habitat changes.**Objectives:**Collect, register and identify the species of small mammals present in a coastal Atlantic Forest fragment at São Camilo Environmental Station, representing a transition area between the restinga formation and the ombrophile forest, in the Municipality of Itanhaém, SP.**Methods**: We used two different trapping methods, withthe sampling effort of 1246 trap/nights, plus camera traps, to inventory the species of small mammals, during the first semester of 2013. **ResultsandDiscussion:**Fourteen individuals belonging to 7 species of small mammals were registered. From these, 4 belong to the order Rodentia, while 3 are marsupials. The species of rodents are (nº of individuals in parenthesis): *Sciurus ingrami* (1), *Oecomys catherinae* (2), *Hylaeamys laticeps* (2) and *Oligoryzomys nigripes* (3). The marsupials registered are *Didelphis aurita* (2), *Marmosops incanus* (1), and *Monodelphis americana* (3).Pitfall traps were more successful in capturing the species then live traps. At least nine species of marsupials and twelve native species of small rodents were expected to occur in the area, based on the published results from other inventories conducted in the same region, but in other altitudes and physiognomies. Some common species of Atlantic Forest small mammals that were expected to occur in the area, such as the water rat *Nectomys squamipes*, and the four-eyed opossum *Philander frenatus* are probably present but were not registered. Sampling at São Camilo Station during at least a complete year, would certainly result in species richness more representative of the small mammal fauna present in this area of the Atlantic Forest. Studies directed to the inventory of bats and medium and large sized mammals are still lacking for the study area.

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8.29 Characterization of the*Ochlerotatus scapularis* populations (Diptera: Culicidae) using markers: COI, wing geometric and genitalia

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Introduction: *Ochlerotatus scapularis* is a neotropical vector of filariasis and arbovirosis. Cases of domiciliation and urbanisation of this species have been increasingly reported. Some morphological and behavioural variations have led authors to believe that this mosquito species is actually a complex of cryptic species. **Objectives:** The purpose of this study was to describe the population structure of this mosquitoand test whether it is indicative of a cryptic species. **Methods:** Population samples were characterised using: Cytochrome oxidase subunit-1 (COI) mitochondrial gene (partial), wing geometrics and genitalia morphology. Samples of adult mosquitoes were collected between 1998 and 2011 in four municipalities in Brazil: Tremembé, one sample (TRE); Pariquera-Açu, one sample (PAR); Itaboraí, one sample (ITA); and São Paulo, four samples (PET 98, PET 07-08, PET 11 and BUT). **Results and Discussion:** Among the 147 individuals analysed, 52 COI haplotypes were found and the haplotype diversity was high (0.916). Six haplotypes were present in 69% of the individuals and were shared by most or all of the populations, whereas the remaining haplotypes were less frequent. In addition, genetic differentiation was low ($F_{st} \leq 0.0602$) and estimated gene flow was not absent. Coherently, the wing shape characteristics assessed using geometric morphometrics were polymorphic and suggest an incomplete population structure. Morphological analysis of the genitalia revealed that the claspette filament was intrapopulationally polymorphic and was not indicative of species complex. We concluded that *Oc. scapularis* should be considered as a single polymorphic taxon. Analyses also showed that wing shape and the COI gene evolved during 13 years. These results lead us to believe that *Oc. scapularis* bears a rich genetic patrimony, which may confer a broad adaptation capacity to this species. Implications of such genetic richness on vectorial capacity, plasticity and ability to exploit urbanised areas should be included in the next stage of the investigation of this species.

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8.30Reproductive behavior of *Philodryasolfersii* (Lichtenstein, 1823) and*Philodryaspatagoniensis* (Girard, 1858) in captivity

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Introduction: The viperid reproduction has been quite documented, however few is known about the reproductive cycle and reproductive behavior of Colubrids and Dipsadids in captivity.**Objectives:**Verify the possible occurrence of agonistic behavior between males, breeding period, courtship behavior and copulation of Dipsadids *Philodryasolfersii* and *Philodryaspatagoniensis*in captivity. **Methods:***P. olfersii*and*P. patagoniensis*kept in captivity in the Laboratory of Herpetology IB. Tocheck the occurrence of agonistic behavior between males, 2 males and 1 female were used to attempt breeding. Were recorded the presence of agonistic behavior between males, behavior and courtship time, behavior and breeding duration.**Results and Discussion:***P. olfersii*– 5 matings of *P. ofersii* were recorded. Non violent agonistic behavior was observed between males. Males moved vigorously in the terrarium, making lateral movements with their body against the opponent. During a few minutes they moved around with their bodies coiled around each other, always flicking their tongues, trying to stay on the dorsal region of opponent with the head ahead of their opponents head. In the observations, this behavior had duration of 18 to 52 minutes (with short intervals). In sequence, only 1 male courted the female. Only in one of the mating attempts both males courted the female, but without any contact between their bodies. The courtship behavior was observed preceding all matings, lasting for 26 to 96 minutes. All matings were observed in July, lasting for 7h27 minutes to 48h57 minutes. *P. patagoniensis* –Were recorded 3 matings. Agonistic behavior between males not was observed. Courtship behavior also preceded all matings that had lasted from 6 to 21 minutes. Matings were also observed in July, and the duration was 9h50 minutes to 24h34 minutes.Although they belong to another family, courtship behavior observed in both species is similar to that already described for genus *Bothrops*. This is the preliminary resultsobtained from a study that wants to investigate the reproductive cycle and behavior of some species of neotropicalColubrids and Dipsadids in captivity.

8.31Characterization of *Aedes aegypti* (Diptera:Culicidae) populations in State of São Paulo

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Introduction: The mosquito*Aedes (Stegomyia) aegypti*, is native from Eastern Africa, but has spread worldwide mainly due to human activities. The presence and dispersion of this mosquito are considered to be a public health problem because of its ability to act as a vector for the dengue, yellow fever and chikungunya viruses. The state of São Paulo, Brazil, displays one of the highest rates of dengue infection in the world, with 190.503 autochthonous cases being reported in the first semester of 2013. The main way to control dengue viruses is reducing *Ae. aegypti* populations, because neither effective vaccines nor antiviral drugs are available.**Objective:** The aim of this study was to assess the population genetics of *Ae. aegypti* in the state of São Paulo. **Methods:** Six population samples were collected during the autumn of 2011 in Santos (SAN), São Paulo (SPA), Campinas (CAM), São Carlos (SCA), Catanduva (CAT) and São José do Rio Preto (SRP). Those six locations were chosen because its overall epidemiological importance: they harbour foci of dengue, they are densely inhabited by humans and interconnected by major highways, which presumably facilitates vector dispersal. Road distances between cities range from 54.9 to 468.4 km. Two hundred and ten individuals (35 per location) were analysed using eight DNA microsatellite *loci*, one mitochondrial gene (COI) and wing geometric morphometrics. **Results and Discussion:**We detected significant genetic ($0.016<Fst<0.114$) and phenetic ($1.93<DM<4.24$) differentiation between the *Ae. aegypti* population samples.Gene flow does not appear to be high among populations. Positive correlation between genetic and phenetic pairwise distances was detected ($r=0.72, p=0.002$), indicating that biological markers were congruent. Neither the genetic nor phenetic distances were correlated with geographical distance. Although six COI haplotypes were found, only one was shared by all populations, supporting the hypothesis of low gene flow among them. SAN presented the higher number of haplotypes (6), being that two were private. Taken together, the phenetic and genetics analyses revealed that *Ae. aegypti* from São Paulo State is populationally structured. The existence of connecting highways did not guarantee extensive gene flow among locations. The SAN population was the most phenetically and genetically distinct and polymorphic, which is consistent with the fact that SAN contains a seaport that could act as a point of entry for foreign *Ae. aegypti*. The results of this study may be useful for developing a more effective vector and dengue control in the state of São Paulo.

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8.32Wing sexual dimorphism of pathogens-vector culicids

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Introduction: Sexual differentiation in Culicidae and its implications on epidemiological importance have been extensively studied. Although only females bear blood-sucking organisms and have vectorial capacity for pathogens, they lack conspicuous taxonomic diagnostic characters in some species. Mosquito wings also play sex-specific roles, i.e. courtship sound production by males and precise appetent flight of females. Even though, wing sexual dimorphism (SD) is poorly investigated. **Objectives:** Aiming to further the knowledge on this filed, we used geometric morphometrics to comparatively characterize the SD of eleven Neotropical medically-important culicids: *Culex quinquefasciatus, Culex nigripalpus, Aedes aegypti, Aedes albopictus, Aedes scapularis, Anopheles darlingi, Anopheles albitarsis, Anopheles homunculus, Anopheles cruzii*, *Anopheles triannulatus* and *Anopheles strodei*. **Methods:** Females and males of the eleven species of Neotropical medically-important culicids were collected between 2007 and 2013 in three Brazilian states: São Paulo, Minas Gerais and Rondônia. For analyze intraspecific sexual dimorphism, right and left wings of each individual was detached from the thorax and mounted with Canada balsam between a slide and a coverslip, digitalized using a Leica S6 stereoscopic microscope and the coordinates of 18 landmarks represented by vein intersections obtained using TpsDig version 1.4. **Results and Discussion:** The amount of SD, estimated by nonparametric shape disparity, variated according to the species and was not correlated to phylogenetic relationships. Additionally, SD levels did not correlate to the habitat (sylvatic or urban) and appeared to be species-specific. *An. darlingi* presented the lowest SD score (4.7) whereas *Cx. quinquefasciatus* reached the highest score (SD>34.0). Remarkably, its score was nearly three times the mean SD score of all species. We hypothesized that such discrepancy is partly due to the hostile habitat of *Cx. qunquefasciatus* (highly polluted urban river). Phenogram of wing shape including the two sexes and all species revealed that only the cluster of females was topologically similar to the phylogenetic tree, suggesting that male and female wings undergo distinct evolutionary histories. Pragmatically, sexes and species can be diagnosed by wing characters. Phylosophically, we should further investigate the possibility of Darwin´s sexual selection on wing traits.

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9. Education and Science Dissemination

9.01 Educational visits at the Butantan Institute, 'Places of Learning: the school gets out of school Program'

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Introduction: The 'Culture is Curriculum Program' is a partnership of the Secretary of Culture and, Secretary of Education of São Paulo, promoted by of Education through the FDE (Foundation for Education Development) and different institutions that provide educational visits for schools in varied areas. The goal is to print a new relation among the schools and the cultural institutions in the State of São Paulo. The project assisted in this program is known as "Places of Learning: School gets out of school." **Objectives:** To evaluate the previous preparation of students and the use of program material made by FDE before visiting the museums of the Butantan Institute in 2011. **Methods:** Museum educators, teachers and students filled a questionnaire after the visit. The questionnaires were about the profile of the group and the development of the visit and their preferences. The teacher should inform about the previous preparation with (or without) the material provided by the program. **Results and Discussion:** The total number of visits at Butantan museums was 80 and for each visit a questionnaire was answered by a museum educator totalizing 192 questionnaires distributed as follows: 60 completed questionnaires in the Biology Museum, the Museum of Microbiology 80, and 52 in the History Museum totalizing 2,711 students in 8th and 9th grade. The investigation has questioned the use and knowledge of educational support material offered by the program from the teacher, asked about receiving or not this printed material and DVD at school. In the Biology Museum, 98.3% of teachers or tutors responded that they did not receive the material; in the Museum of Microbiology 72.5% did not receive; and in the History Museum 96% of them did not. We conclude that most of the visiting professors had no contact with the program material, a fact that reflects directly in the preparation of the visit. When teacher were asked if they prepared the students for the museum visit whether or not there prior preparation group, 95% of the Museum of Biology respondents claimed not to prepare the group prior to the visit; In the Museum of Microbiology 67.5% of respondents did not prepare the group and in the case of the Museum of History 94% of respondents claimed not to prepare the group. The data indicate that the groups do not prepare for the visit and come to the institution as they would for any leisure activity. The visits are not prepared in advance in school, which undertakes educational work in a certain way. One of the goals of the Program is precisely strengthen teaching through new forms and possibilities of development of curricula, and the non-preparation of the group hampers the performance of the visit in the aspect of their educational potential.

9.02 Visual Identity Program Vacations at Butantan, a small part of institutional history through their characters

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Introduction: The Butantan Institute, since 2011 performs the program *Vacations at Butantan* in which visitors have the opportunity to explore different themes of science. The activities provide a contact with the institutional history and promote an enriching experience for learning and knowledge of the public about the scientific themes developed in Butantan. In 2013 verified the need to create a visual identity for the program, the cultural area thought on relive theinstitutional memory by some prominent researchers to be the representatives of this identity, who have devoted their lives to work in Butantan, reinforcing the collaboration of these people to the history of public health of the country. **Objectives:** Raise and describe the history of the characters chosen to represent the visual identity program called *Vacations at Butantan*. **Methods:** We created a working group for art production and research to selected pictures to compose the visual identity. These pictures represent researchers, who are not alive but were selected because they were very important at different work areas of the institution. The historical survey of the characters was conducted by consulting different sources, including: library books of the institution, PhD thesis, the collection of memories of the institute,researcher's books and other materials, as well as interviews with key researchers. **Results and Discussion:** Over the editions of the program Vacations at Butantan staff of the cultural area identified the need to develop a visual identity that corresponds to the proposal and that is different from other activities. Through the survey and description of the history of these personalities, each activity associated with the program received one of these characters to represent it. This way, it was possible to show the importance of some institute researchers and let the public know about the lines of research developed by the institution. One of the outcomes of this research refers to the process of surveying the history of the researchers selected as the reference sources are scarce and into the files of the Institute there is little information about the researchers. Given this, it is difficult to trace a path from each institutional character, without for example, to know in which laboratories worked and the different research that they developed. Therefore, it is necessary to think about the access to and to disseminate of the rich history incorporated within public institutions in a systematic and organized way. The creation of the visual identity was important to consolidate the image and purpose of the event. Actions like this contribute to spread abroad the history of the national scientific development.

9.03 Science, Technology, Innovation and Development-the role of research institutions and the Brazilian health system: an experience in the academic extension course

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Introduction: Scientific dissemination is an important issue in the scientific and technological research funding agenda. A portion of the budget of main thematic projects, as CEPID or INCT is directed linked to these activities. The LEHC in this year offered a course which objective was the discussion about the role of research public institutions in the actual context of Brazilian science. **Objectives:** This paper presents this experience and some reflections about repercussions and possibilities that opened to researchers from Butantan Institute and other institutions. **Methods:** Descriptive study based on register of leaners sections from CDC, LEHC and didactic material for the course,which was based on LEHC´s researcher two experiences: one over B Hepatitis's vaccine development, and another about technological transference on *Influenza* vaccine.The course had 22 previous subscriptions made and nine effective, the schedule of 20 hours, in a week period. Although there were ones from others institutions, the majority of learners were composed from *Butantan*´s professionals and postgraduate fellows. The program applied was: 1. health systems in Brazil and other countries, epidemiological transition, Public Health policies and science and technology demand. 2. Concepts of Science, technology, research, development and innovation. The Pasteur´s Quadrant, Patents and Copyrights (an international overview). 3. Research institutions in Brazil. 4. Research Policies, induction, funding and support institutions. 5. Social and political determinants in Science and Technology. **Results and Discussion:** The authors' main perception was that young researchers were searching for knowledge about the origins of Brazilian research institutions; agencies and mechanisms of funding; the industrial and commercial copyrights issues, whose bureaucratic and economic costs were unknown. We have noted that the relationship between State, Society and Science was unclear for the learners, which understand that the science is disconnected from these other dimensions. The assessment performed after the course was positive and pointed a lack of familiarity with these themes, and also an enormous interest from these professionals about these questions as well as to understand the *Butantan*´s history from its relations with Brazil´s science and technology in different contexts. Extension courses are an important way for scientific dissemination even for professionals, and attracting people to share scientific points of views and helping to demystify misconceptions about the role of scientists and myths related to the neutrality and the autonomy of science and scientists.

9.04 ProjectRecords Managementat InstitutoButantan: challenges ofmanagement andaccess ofdocumentsin science and technology

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Introduction: The Núcleo de Documentação of Instituto Butantan, responsible for the organization, preservation and the diffusion of institutional records, has worked on creating a institutional acquisition policy. This process depends on the involvement, above all from the Production and Research fields responsible for the institutional production of "end" documents (linea-function). **Objectives:** Identify the institutional documentation of permanent character, under responsibility or not of the Núcleo de Documentação; discuss and conceptualize the term *scientifical documentation*, within the parameters discussed nowadays among historians and archivists; systematize normatives that allows guard and disclosure of science and technology acquisition, according to the current archivistical legislation.**Methods:** In order to sensitize the great four institutional fields – Cultural, Scientifical, Production and Administration – general meetings were organized on the Project and the Information Access Law, wich participated, besides the Núcleo de Documentação team, partners from Departamento de Gestão do Sistema de Arquivos do Estado de São Paulo – SAESP and from Secretaria de Saúde de São Paulo. In those meetings, representatives from every institutional field where present: fundamental collaborators of permanent records identification. Alongside this first moment, we have sent to the 42 present units, a diagnosis survey which objectives the produced acquisition examination at each unit, the storage and boxing, access, guard and discard definitions, record type identification, dates and quantification of documentation. **Results and Discussion:**From the fifteen surveys refowarded to the Núcleo de Documentação, where distinguished some expressed questions inside the most frequent doubts related to record types - documents that meet common features in content or in technique, such as processes, reports, letters, etc.; inclusive dates - chronological identification of documents; guard criteria and mainly on classified confidential documents access produced by the Laboratories, which demanded a more effective accompaniment from the responsible archivists. In order to address the questions we are most frequently asked, the archivists have revised the questionnaire that, from now on will be done with accompaniment. With this redesign will be possible to make the relationship between the common questions expressed and the absence of internal policies that focus on the guard and the disposal of documents managed in these laboratories.

9.05 Organization of data holdings in research on health and popular treatment in Belterra/PA and Juquitiba/SP under INCTTox

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Introduction: Since 2008 fieldworks to Belterra/PA and Juquitiba/SP have been made to research the health history and popular treatments of these locations. The team of this work collected data and documents in different typologies. To turn these documents into a research source it must be organized, tabulated and managed. The collected information must be in a logical way for future search, works and publications by turning the different kinds of information into a data holding. **Objectives:** Due to the diversity of the collected documents we purpose to organize the textual, audiovisual and iconographic sources to a forthcoming propagation and for critical analysis of the ongoing research. **Methods:** Initially the sources were only chronologically classified so we had to recognize the origin of the audiovisual and iconographic collection using the reports done in the research. First we distinguished the research'sregistered localitiesand the actions promoted by the team. Then we created search zones in specific virtual folders within a mass storage device.Now, there are different ways to access the information according to the researchers' needs. **Results and Discussion:** The following folders were created to structure the search zones: Localities; Projects; Biodiversity. In the first there are all the digital files divided by date and locality where we can find all kinds of information the involved characters (local leaderships, interviewees, health professionals, educators and collaborators), kinds of actions promoted by Butantan (educational and cultural, research worksand prospections) and locality (Amazonas, Pará and São Paulo). In the second only the records of the actions performed with the local dwellers; there are 39 interviews, 11 workshops, 4 readings of the Consent Term with the local leaderships in an easy way to find it. In the third there are the photographs and videos of the wildlife from the researched localities. We filed the printed sources related to the research in an appropriate location available to the team. The files were classified as: Maps; Newspapers; Magazines; Books and Thesis; Divers, following the alphabetical order.There are about 150 different titles to consult. Until now, we cataloged 14420 photographs and 1017 videos, collected by the team in 11 fieldworks. Further, we developed a registration form of the collaborators with their photograph as a way to a better knowing and searching of the people involved in the research.

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9.06 Use and aplication of turtle shell of Chelonian as a tool for knowledge and awareness of the visitors of the Instituto Butantan

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Introduction: Since 2008, the *Pátio Casa Vital Brazil* (PCVB), Herpetology Laboratory, keep the specimens of Chelonia received at Butantan´s Animal Reception. The *Trachemys* (tiger water, fresh water turtle) (T. *dorbignyi* and T. *scripta elegans*) are kept in a swimming pool. Nevertheless, two young specimens of *T.dorbigyi* (Brazilian Tiger of water) had recently died, which gave the idea of using their carapace (the top or dorsal shell) and plastron (a bottom or ventral shell) to become educational material. **Objectives:** In order to promote projects between the scientific and cultural areas, the proposal seeks to obtain pieces with artistic imprint representing species and their behavior in nature, so the visitors can touch and interact like it is the animal itself among others activities. **Methods:** After the death of the specimens, carapace and plastron remained connected as one, once the insides were eaten by other *Trachemys* in the enclosure, naturally stripping their inner parts. The turtle shell were cleaned in a solution of water and sodium hypochlorite and wrapped in gauze with coarse salt, then received a lubricant to dry completely before the artistic intervention. To support the confection of the piece, were used photographic references and observation of animals in situ. The carapace and plastron had its interior filled with clay, supporting the handmade head, tail, legs and feet to be built. Once dry, the clay was sanded and painted to look as close as possible to the *Trachemys´* original color.Yellow stripes were scattered along the body, with special emphasis to the yellow ear that characterizes the brazilian species. *Biscuit* was used to produce the tail, because it showed better sustainability and acceptance to the *gouache* paint application. **Results and Discussion:**The single prototype that was finally prepared from the *Trachemys ´* carapace and plastron presents consequently,legs and feet to simulate the specimen´s movements, and head, shaped and curved with volumes that appropriately represent the turtle's anatomy.The prototype was already tested, and positively approved by the *Butantan para Todos*group, used for an accessibility workshop promoted in the 'Vacation Week' activities of the Institute.This final piece, summed up with the artistic and playful intervention presents itself as a completely educational tool version for the live animal, offering to the public, a multisensory experience. In addition, there is a great effort to extend this work and artistic technique, to other specimens that have been preserved, dead and frozen, by the PCVB, for future educational and playful activities besides others actions of culture and science.

9.07 Butantan para Todos: Sense Workshop

Foronda L[1], Freitas AC[3], Tozetto Neto L[1], Arrabal EM[2], Milanelli R[2], Mezini A[4], Nascimento CV[1], Saito CA[2], Sato LM[2], Lemes DCE[4],Turcarelli T[1]
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Introduction: The group *Butantan para Todos*, established in order to promote cultural accessibility in the Butantan Institute, created an approachable multisensory education activity: *Oficina dos Sentidos* for the "Vacations week" in 2013 edition. This activity was thought to be accessible for all publics and was developed upon the Vacations Week´s theme: "Nature and its forms" **Objectives:** To provide interactive experience within a cultural institution and reaffirm its collections may be used by the visitors beyond the contemplative observation. Experience instigates multiple senses beyond vision, such as touch, smell and hearing, providing an insight different from the usual, where the vision is usually felt more valued over others. It also aims to include in the agenda of the institution the issue of inclusion by expanding the possible means of enjoyment of objects. **Methods:** The activity provides a route to be experienced by visitors, divided conceptually into two environments: terrestrial and aquatic. The participants enter the route blindfolded, accompanied by an educator who guides and provides the interaction with the exhibits and the environment. The objects are disposed in long tables to touch and to interact (fauna and flora) according to the environments represented by taxidermy animals, art reproductions and handmade: scorpions, spiders, snakes, turtles, armadillos, fish, shrimp, corals and plants. This route is like a "U" shape in a fixed space, isolated, in order to control and seal the route taken by visitors. **Results and Discussion:** There were 288 participants / visitors, including children and adults, who touched and interacted with the objects produced by the team and who have demonstrated understanding of the contents. Only children under four years old abandoned more frequently and caused more difficulties to the educators in mediating. In addition, a child in wheelchair with cognitive impairment participated and demonstrated a good comprehension of the workshop. The evaluation showed that the materials employed were effective for readability for touch and the tactile collection was consolidated representative of the Institute.The group intends to continue this project with the inclusion of all kinds of audiencesin special activities (for example: Vacations Week) and also as permanent actions inside the museums (exhibits and education).

Supported by Fundação Butantan

9.08 The educative potential of the frottage in the exhibition "The Giant World of the Microbes"

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Introduction: Due to demands of visitors from different ages, especially children accompanied of their relatives, and the need to adequately assist them, the Museum of Microbiology producedan exhibition addressed to children audience called "The Giant World of the Microbes", which is composed ofexhibits that deal with microbiology subjectsthrough interactive games, videos and drawings. The frottage was selected among the exhibits for this study. This exhibit consists of metal reliefs of different microorganism images, which can be engraved in a paperusing crayons. Drawingis a way of representation that exercise children imagination and creativity and characterize phases of children development. Therefore, the frottage can be considered an object that promotesthismannerof representation. **Objectives:** Analyze the potential of the frottage for helping the comprehension of different microorganism forms by children from 4 to 10 years-old visiting the exhibition "The Giant World of the Microbes". **Methods:** Twelve children who visited the exhibition were interviewed in July, period of great frequency of children due to vacations. They were asked which apparatus of the exhibition they most liked and why, whether they liked the frottage, which image of the frottage they found more interesting, whether they were able to identify differences among the drawings and whether the drawings have the same form and size than the actual microbe. The interviews were recorded and transcribed to analyze the data. Observations about how the children interacted with exhibits were also done. **Results and Discussion:** The results showed that, although the intended public of the exhibition was 4-6 years-old children, older children from 7 to 10 yearsalso presented significant experiences with the frottage, which was the second most preferred by the childreninterviewed. Besides, all children answered that they like to use it. The motivations were related to the act of drawing as well as the opportunity of knowing the microbes. The virus was the most preferred draw (4 responses). Independently of the favorite microorganism, the most important aspect of children answers was the recognition of the diversity of microorganism forms, even relatingthem to objects of their everyday lives. However, no relationship was done between the forms and the groups of microorganisms.The results also revealed that children have difficulty to understand the concept of scale, since they were not able to distinguish between magnifying lens and microscope as equipment to observe microbes when asked about the size of microbes. In conclusion, the frottage reaches the goal of showing different forms of microbes and was efficient to be also used by 7-10 years-old children.

Supported by Fundação Butantan

9.09 Evaluating the Experience of the Public in naExhibit on Biodiversity: The Attraction of Dioramas and Perception of the Central Concepts by the Visitors

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Introduction: In 2012, the Museu de Zoologia of the Universidade de São Paulo (MZUSP) presented a temporary exhibition in the Estação Ciência (USP). Named "Biodiversidade: Fique de olho!", the exhibit proposed a reflexion upon the challenges represented by the contemporary threats to the Brazilian biodiversity. **Objectives:** The main purpose of this work is to evaluate, through the observation of the behavior and interviews conducted with an aleatory sample of the visitors, some aspects of the experience and perception of the public, and the understanding of the main biological concepts represented in the exhibit. Data was collected during September and October, in 2012. **Methods:** The sample was composed by spontaneous visitors, with fifteen years or older, Portuguese speakers. The course of each chosen subject through the exhibit was registered and drawn on a map, and subsequently these visitors were interviewed to collect demographic variables and answers related to their cultural habits and perceptions regarding the displays. All their actions along the exhibit were recorded (e.g. taking notes, conversations, reading of the legends or texts, taking pictures, watching videos and playing the games), combined with the amount of time spent at each point. **Results and Discussion:** The results from the final sample of 65 visitors show that most of them are female (69%), between 20 and 34 years old (54%) and with higher educational level (61%), mostly families (70.6%). The visitors took, in average, 14 minutes to go through the entire exposition. The analysis of data on timing and tracking indicated that the dioramas are the displays that draw most of the attention and where the visitors spent more time. Texts, videos, and games were the displays with lower attention capacity, although with higher retention power. Results of the analysis of the interviews confirm that the dioramas, taxidermized animals, original skeletons and replicas are considered the most interesting elements of this exhibit, when compared to the games, videos and texts. As for the design and arranging of the exhibition, the results indicate that the path to be followed was not clear to the audience, since 50% of the visitors came in through the exit and 88% left by the entrance. The analysis of the data from the interviews indicates that the central concept of the exhibition, which was to propose a reflection on Biodiversity and the modification of natural environments, was not properly understood by the audience, although words like conservation and preservation are common in visitor answers.

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9.10 Characterization ofan Education activity usingLive Animalsinthe Museu Biológico

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Introduction: TheMuseu Biológico(MB) isthe oldest Museum in the Instituto Butantan, and historically the most visited from the three museum situated in the Park. It present to the public displays and information related to snake biology and accidents with snakes, and the exhibit is constituted of dioramas with live animals. The docents of this museum developed an activity in order to promotea close interactionbetweenthe spontaneouspublicand the exhibit and main theme presented by this Museum. This activity allows the contactbetween the visitors and someof the animalsthat compose the collection. Called"Pop-up", the activity isheldon weekendsand holidays, during half an hour in the morning, andit is mediated bythe Museumdocents. Eachmontha different group (among reptiles, turtles, frogs and invertebrates) ischosen to be presented to the public in this activity. Besides allowing the visitor to "feel" the animal, another objectiveis to create aspace to shareinformation aboutits biology, providing apositive interaction. Such experiencemaybe amodifierof attitudes, beliefsand behaviors. **Objectives:** Thiswork seeks to characterizethe publicthat participated in the"Pop-up" activities, establishing theirprofile in terms of ageand gender. **Methods:** During thedevelopment of the activity, the attendees were counted and categorized as childrenup to 12years old,young people(12-19 years old), adults(20-65 years old) and elderly(over 65 years old). The last threecategories werefurther subdividedaccordingto the gender. Datawas collected during the monthsof May, Juneand July2013, when school groups were not so common among the visitors, as only spontaneous groups were included. The animal groups presented to the public during these three months were, in order, arthropods, amphibians andsnakes, one at each month. **Results and Discussion:**Duringtheperiod analyzed, 1250visitorsattended theactivity, representing13% of the total number of visitors of theMuseu Biológico during this same period. Frequencyobservedamong participantsare: adults(men and women)55%, followed bychildren 36%, equivalent to91% of all the participants. The dataindicatesthat most of the attendees werefamilies, composed ofchildren under 12years old andadults. Young peopleand adults not part of a family groupwere not well represented in our sample, therefore constituting a group that should be focus of new activities to be proposed in the future.

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9.11 Analysis of the comments and suggestions present in the Visitors' Books of the Museum of Microbiology of ButantanInstitute

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Introduction: The improvement of the educational action of a museum joins the assessment process of the visiting public and their relationship with the exhibits, this being one of the most valuable steps in organizing new exhibitions. Evaluation should be a process, not just one-off event. Among various methods, the book of signatures can collect different information about the public and offers a large sample, and is therefore the focus of this work. **Objective:** Evaluation of educational actions and exhibition of the Museum of Microbiology based on categories of most recurrent suggestions and critiques identified in the books of visitors of January 2009. **Methods:** The responses were tabulated, reviewed and classified into categories and subcategories, which were determined based on the following criteria: presence or absence of comments, when there were comments, they were separated into positive or negative, with or without suggestions about the Museum as a whole, staff of the Institute, the long-term exhibition, the expography, infrastructure or other activities offered by the Museum. The reason for the choice of the period analyzed was the great movement of visitors in the Museum in January, because of school vacations, and changes in the layout of the exhibition made in the previous year. **Results and Discussion:** 39.34% of the respondents did not fill the field "Comments, suggestions and critiques" of the table or answered "no" or "nothing", possibly because these visitors have enjoyed the visit and found no need to leave a comment about their experience. Among the 722 responses that had filled the field, 671 (92.94%) were compliments, while only 23 (3.18%) were negative critiques or suggestions, of which 65.22% (15 responses) belonged to visitors with higher levels of education. In general, the content displayed in the Museum and didactic approach of the exhibition were praised by visitors. The evaluation of the comments indicate that the majority of the public sampled felt themselves as part of the Museum by requiring employees to take care ofthe museum and suggest increasing of content, reassessment of monitoring, greater dynamism and increase of interactivity in the exhibition.

Supported by Fundação Butantan

9.12 Evaluation of the activity Open Laboratory from the Museum of Microbiology

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Introduction: The Didactic Laboratory of the Museum of Microbiology was designed to provide programmed activitiesfor schools. On school vacations, the museum offer an activity called Open Laboratory, an opportunity to the spontaneous public to know the space and its equipments. In the last edition, the educational staff felt the need to understand how the public see this action, so an evaluation protocol was elaborated. **Objectives:** Characterize the ethnographic profile of the public understand if this public was composed by regular visitors of the museum, how familiar is the audience with laboratories and how frequently the public thinks the museum should offer activities like this. **Methods:** The survey was applied to the public that had volunteered to answer after doing the activity and being informed about the survey. In general, people were accompanied by a group and all of them could express their opinion, although the ethnographic data was gathered from only one person. **Results and Discussion:** During the four days in exhibition, the museum received in its laboratory 1508 visitants, 62.5% from the total visitors on the period. The number of surveyed visitors was 106, all were accompanied by groups, amounting 396 persons.The answers were gathered mostly in the afternoon (70%), this data is in agreement with the museum records of visitation that characterize this period as the most visited. Among the respondents, 38% were aged between 30 and 44, whereas 27% from these had completed college. Each group had, on average, 3 people of which 62% were accompanied by their families (grandparents, aunts, uncles, cousins, etc) and 42% were with their parents and/or sons. When asked if they had entered into a laboratory before, 62 people (58%) answered no. Between the ones who answered yes,35% said that they had gone into a laboratory in the school and/or faculty. Among the 80 respondents who had never been to the museum 76 said that they would come back to do an activity like that. The visitors considered that the activity had great relevance to their daily lives, totaling 68 that could establish an entire relationship between the activity and their daily lives. Despite the activity has occurred during the week, 65% of the respondents said that they would like the museum provides this kind of activity on weekends, reinforcing the need for the museum streamlines its activities to the spontaneous public. By performing this evaluation, it was also observed that the museum as a whole can provide a closer approximation of the spontaneous visitor with the world of science through entertainment by proposing educational actionsin a pleasant way in its laboratory.

Supported by Butantan Foundation

9.13Evaluation of the Extension Course "Science museums: a place forinformal education", promoted by the Museu Biológico do Instituto Butantan, together with the São Paulo Zoo and Museu de Zoologia da Universidade de São Paulo

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Introduction: The Instituto Butantan offers university extension courses open to professionals from various fields, in order to stimulate the interest in science, culture and technology, providing updated content from various themes. Some of the courses directed to school teachers and educators discuss the science popularization and pedagogical practice in spaces like museums and science centers, devoted to informal education. Through a partnership with the São Paulo Zoo and the Museu de Zoologia da Universidade de São Paulo,in 2007 the Museu Biologico created a course with a pedagogical objective common to the participating Institutions, presenting them as spaces of informal education in which the animal is the main element of the exhibits and seeking the instrumentation of teachers and other education professionals while visiting a museum or science center. **Objectives**: This study aimed to identify the expectations of the target audience of the course and its outcome based on a evaluation form presented to the attendees at the end of the classes. **Methods:** Questionnaires were composed by objective and discursive questions, and filled by 10 participants in 2013. Individual responses were tabulated on spreadsheets and from them, categories were created for analysis in order to identify whether the objectives of this course were met. **Results and Discussion:** The profile of the participants is female, between 20-30 years old,graduated teachers and educators. The analysis showed that in general, participants evaluated the course in a positive way. In the 2013 edition of the course, the participants rates as positive the heterogeneity of the audience of attendees, with a diversity of professionals working in the field of informal and formal education, which allowed the exchange of experiences and new methodologies. The most positively evaluated aspects, in their opinion, were the participation of the coleagues during the course, discussion of the practical aspects that could be implemented in the classroom, the ability to integrate theory and practice, and the development of a project. Points considered average: the absence of media information about the course, location and organization of spaces and schedule of activities proposed. As also evidenced by the former evaluations in other years, the results corroborate the expectations and opinions of the participants: search for update in didactic content, self-improvement and sharing experiences.

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9.14Waste Management Program at Instituto Butantan: Development of a practical guide on waste disposal

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Introduction: The directory of Instituto Butantan (IBu), committed to environmental issues and legal compliance, has implemented a Waste Management Program, aiming at reducing waste generation and promoting proper disposal and safety, minimizing the negative impacts on the environment and in public health. The adequation of waste disposal at IBu to the National Solid Waste Policy as well as to other environmental laws would run easier and smoother with the help of easily readable, fast access educational material addressing IBu's specific needs. **Objectives:** To develop a practical guide on waste disposal that can be used in all areas of IBu and of São Joaquim Farm, respecting the environmental regulations, proper waste management and occupational safety. **Methods**: A survey of the waste generated in all Butantan's areas was performed. Next, a multidisciplinary and multi-sectorial team of professionals, taking into account Federal, State and Local laws that rule waste disposal, elaborated a guide containing information and procedures for waste management from its generation to the final disposal. **Results and Discussion**: In addition to the procedures regarding proper disposal, the guide includes information about the laws involved in this work, the classification of health service waste and personal protective equipment guidelines. To help consultation, wastes of different nature were separated in groups: Infectious (including animal carcasses), Chemical, Radioactive, Ordinary (recyclable and non-recyclable) and Special (batteries, electronics, lamps, ink cartridges and toners), and were discussed in separate chapters. The content focuses specifically on the waste generated at IBu and at São Joaquim Farm. Waste types not generated at the IBu complex, though described in law, were not discussed. Each chapter considers procedures for segregation, packing, identification, and waste transportation. The guide was then opened to public consultation in order to obtain input from the internal community. The completion of a manual that contains procedures related to waste management, with the contribution of the users themselves, establishing the standardization of disposal of the different groups of waste to minimize environmental impact, improve worker safety in the institution and respecting the law, is a pioneering and innovative initiative that gives IBu distinction in sustainability and environmental responsibility issues.

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9.15 Teachers' conceptions of practical classes and the role of the Didactic Laboratory of the Museum of Microbiology of Butantan Institute

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Introduction: Science education considersthe experimentation essential to scientific learning. For a long time, laboratory activities were used for demonstration of theories, but currently this kind of activities began to be associated with the increase of student's motivation, the improvement of scientific knowledge learning and the hypothesis testing. In this context, the Museum of Microbiology of Butantan Institute offers 5 laboratory activities (three sequential modules, the DNA workshop and the Micro World workshop) in the Didactic Laboratory (DL) in order to encourage teachers to conduct practical classes in their schools, even if the school has no laboratory or equipment, and thus also encourage students to question, create hypothesis and search problem resolutions. **Objectives:**Identify the conceptions of practical classes for science teaching of high school teachers who use the Didactic Laboratory of the Museum of Microbiology. **Methods:**For data collection, interviews were conducted with a group of nine teachers who followed with their students the practical activities undertaken in 1st module. These interviews were transcribed and analyzed along with the bibliography that investigates the use of laboratory classes in science teaching. **Results and Discussion:**We identified that the majority of teachers consider the practical lessons important to students to understand and assimilate the theories seen in classroom and in textbooks. This conception agrees with some studies that show the traditional and simplistic view of practical classes, where the emphasis is on proving theories and not on learning by questioning. Most of the teachers were not clear when answered about the need of infrastructure for preparation of practical classes, because, although they have said that it is possible to do experimental activities with simple materials, they also said that the main factor that prevents the practical classes is the lack of infrastructure and equipment. In these cases, it could be possible that the activities in DL were replacing activities in school. Therefore, considering that the main objective of these activities is to encourage teachers to conduct practical classes in their schools, it is difficult to say if this goal is reached and if there is continuity in school of the activities initiated in DL. Further investigationswould be necessary to address these questions.

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9.16Learning Games: a new educational practice for the guided visits to the Biological Museum of the Butantan Institute.

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Introduction: The Museu Biológico of the Instituto Butantanreceives 140 thousand guests each year, and twenty percent are school groups (data from 2012). Students and their teachers come to the Museu Biológico looking for a source of knowledge that is different from their schools and institutions of origins, as well as new experiences. The exhibit at this Museum comprises biodioramas showing a scenario and containig living animals, allowing the visualization of the species habitats. The terrariums that form these displays include exhibit labels containing general information about the biology of each species, such as feeding habits, characteristics of the reproduction and maps showing the geographic distribution. Still, a significant number of visitors usually ask for more information from the docents regarding the biology and ecology of the species displayed. Therefore, the team of docents of the Museu Biológico developed a game to be played at the end of the guided visits, thus providing new opportunities for the visitors to learn about food, predation and ecological interaction between snakes and other animals. **Objectives:** This work showsthe new game developed by the docents of the Museu Biológico designed for visiting school groups, addressing the ecological relations of the animals on exhibit. **Methods:** We conducted areview of the literature on the use of games in science and biology teaching and therole of games in the context of the teaching/learning process. Additionally we gathered information related to the biology of the snake species in the exhibit. We used Corel Draw Version 12 for design of game. **Results and Discussion:** Being ludic and creative activities, games allow students to learn more about a specific topic, and, in our case, strenghtening the educative potential of the Museu Biológico in the efforts to bring to the public the knowledge about biology and ecology of snakes. Therefore we developed the following elements to compose the game: a board, with a path to be followed along the shape of a snake, and 50 cards, with three different types of information (feeding, predators and card-question). The game developed employs mimic, questions and answers, and offers a moment during the visit in which students, teachers and docents can exchange ideas about the animals observed in the exhibition.Comprising the museum as an institution devoted to the generation and dissemination of knowledge and social interaction, this new proposal becoming an alternative in the process of teaching and learning in Museu Biológico.

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9.17 Evaluation of the audience satisfaction regarding the videos "Nosso corpo: Os germes e o que causam" and "Nosso corpo: Como cura a si mesmo" exhibitedat the Museum of Microbiologyof Butantan Institute

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Introduction: In orderto take a distance from the traditional education at classrooms, the videos have been incorporated into teaching in educational institutions. The video mobilizes sensations and feelings, making memories more lasting and easily retrievable. When properly used and produced, the video becomes a potential educational tool. However, if there is no proper contextualization, the video becomes distant and do not arouse the viewer's interest. At the Museum of Microbiology two didactic videos are presented to children audience: "Nosso corpo: Os germes e o que causam" and "Nosso corpo: Como cura a si mesmo",directed at children between 5 and 12 years old.These videosaddresses issues related to microscopic life in general and aim to increase visitors' interest in the world of microbiology. Because of the videos were produced in 1997, it is necessary to verify whether they are still suitable for learning, considering both didactic aspects and quality of the video production and language. **Objectives:**Analyze the opinion of adult audiencevisiting the Museum of Microbiology with children,about the videos "Nosso corpo: Os germes e o que causam" and "Nosso corpo: Como cura a si mesmo". **Methods:** A questionnaire was developed to each video and the adults, who visited the Museum and attended at least one of the aforementioned videos, were invited to respond it along with the children they accompanied. For the analysis, the categories of Gomes (2008) were used: content, technical-aesthetic aspects, pedagogical purpose, support material and the intended audience. **Results and Discussion:** The questionnaire was applied to 143 visitors. According to the respondents, the images and language of the videos helped children to understand about the main subjects covered by the videos (~ 83%).The subjects were assessed as adequate, clear and sufficient, thus the prior knowledge of children can be considered adequate for understanding the film. Most children said they enjoyed the video (97%), mainly because it is a cartoon and talks about interesting subjectsthat are close to their everyday lives, as they also get sick and hurt. Adults considered the videos appealing to children, yet 33% suggested improvements, especially regarding audio, image and modernization. In conclusion, the results showed that these videos are adequate in terms of contents and pedagogical purposes for the intended audience.Although not emphatically pointed out,the results also indicatedthat it is necessary to update the videos, mainly improving the sound and images used.

Supported by Butantan Foundation

9.18 Microbes: how are they?

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Introduction: For one week during January and July vacations, the Center for Cultural Diffusion and the Museums of the Butantan Institute come together to offer activities for children and adults who come to the Institute during this period. Activities are planned following a central theme. In July of this year, the theme was "The Nature and its forms", for which the Museum of Microbiology offered an activity called "Microbes: How are they?" To most people, the world of microbes is difficult to understand, many of them ignore the fact that there is a plethora of different and varied forms of microorganisms. A great challenge of the Museum of Microbiologyis to approach and sensitize people about their existence. **Objectives:** The activity "Microbes: How are they?" aimed to show, mainly for children, how arethe microbes regarding their forms, diversity and size, as it is not very clear for the general public what means a microbe to be increased 100 fold to be seen. **Methods:** The activity was offeredduring 1 hour for children above4years-old and featured4 interactive cards accompanied by 4 cards of questions about each group of microbes: bacteria, fungi, virus and protozoa. Forms of microbes were selected according to their similarity to macroscopic objects of everyday life of children in order to be compared. In addition, there was a panel displaying the relationship between sizes of common objects,for example, increasing a pinhead 50,000 times means leave it to the size of a basketball. For the development of the activity, each group of maximum 4 children chose what kind of microbe they would like to work (bacteria, virus, fungi or protozoa). Then the cards with questions were shown to the children in order to identify their prior knowledge. The children were instructed to draw microorganisms and then the drawings were discussed, presenting the interactive cards with the pictures and the actual shapes of microbes, which were also compared with common objects in terms of size and form. **Results and Discussion:** We received 97 children who seemed to enjoy the activity. They manipulated all parts of the cards and were interested in working with the 4 groups of microbes. The drawings initiated the discussion on the forms, and the comparison with objects facilitated the mediation. Although the panel was thought to present the concept of scale, the comparison between microbes and objects contributed to deal with this concept. Since it was difficult to apply the activity for children under 5 years who hardly understood the comparisons, the minimum age should be revised. The playful approach helped to hold the attention of children, arousing the curiosity even to visit the Museum of Microbiology, and facilitating the comprehension of those abstract concepts.

Supported by Butantan Foundation

9.19 Audience evaluation of the Museu Biológico (Instituto Butantan)

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Introduction: The Museu Biológico of the Instituto Butantan is one of the traditional references in the exhibition of live animals, primarily venomous, in Brazil. Located in the former stable of the Instituto Butantan since 1966, the current exhibition dated from the early 2000, and received more than 140.000 visitors only in 2012. However, little is known about the public's perception of this exhibition, and specifically, about the venomous animals that are exposed. Thus, In 2013 we conducted a series of surveys with the public in order to better understand the visitor's habits, their perception of the Museum and its exhibit. **Objectives:** To evaluate the perception of the visitors of the Museu Biológico about its current exhibition, aiming to to identify what are the informations transmitted by the displays and the relationship of the visitors with some animals exposed, specifically snakes.**Methods:** During the months of March and April 2013, questionnaires were aplied to a random sample of visitors, after the visit to the Biological Museum. The questions were related to sociodemographic profile, habits related to visits of both the Instituto and other museums and science centers, and nine questions on topics related to the exhibition and its contents. One hundred and fifteeen visitors were interviewed. The interviewees were 15 years old or over, did not belong to organized groups and were Portuguese speakers. The answers were grouped for the analyses using systematics that were employed in other research projects on public behaviour in Museums. **Results and Discussion:** Among the interviewees, the main message of the exhibition refers to the general knowledge about animals, (41.4%). The second topic that appeared in the anwers about the message of the exhibition refers mostly to the conservation of species of animals (33.4%). The theme "Diversity", which according to the curators of the exhibition was the main focus of the displays, was identified by only (11.5%) of respondents. When asked what they liked best in the exhibit the visitors cited first "the snakes" (69%), followed by "scorpions" (10.4%) and "spiders" (6.9%). This result might be related to the aranging of the exhibition, as snakes represents (74%) of the animals displayed. When asked about what the reaction would be if they meet a snake, the answers showed different results whether the hipothetic animal would be in a forest trail or at home.

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9.20 Tactile models of microorganisms and other organic structures as teaching tools

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Introduction: Seeking to contribute to the inclusive activities offeredby the Museum of Microbiology,the educators initiated thepreparation of tactile objectsat the beginningof 2012. The pieces wereelaborated with low-cost materials.They are easy to use andcan aid persons with visual disabilities or not to understand concepts of microbiology. **Objectives:** Enable persons with visual disabilities to know some microorganisms and understand some concepts of microbiology, and make these subjects more playful and dynamic to all visitors.**Methods:**Boardswith reliefs were produced to simulate images of microscope slides and tridimensional models to represent microorganisms, cells and other organic structures. The pieces were selected based on the contents of the museum exhibition and were made manually with materials like Styrofoam, glue, biscuit dough, wool, ink, among others. The following models were prepared: HIV, flu virus (influenza), antibody, antigen-antibody binding, and*Trichonympha*.The boards represented slides with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, with blood cells, with protozoa found in untreated water, with *Clostridium tetani* and with*Aspergillus*. In order to be attractive to visitors with visual impairment and to general audience, all models have contrasting colors, which facilitate the comprehension and identification of structures,besides having aesthetic importance.The pieces were used in school group visits, visits ofpersons with visual disabilities and audience without disabilities.**Results and Discussion:** Observations and testimonials collected randomly from visitors who used the objects showed that these materials, in addition to being simple to prepare, brought positive results when used as mediation tool to teach contents and concepts of microbiology. Some studies indicate that without the mediation of an educatorthe didactic process of using of this kind of objects may not achieve the greatest potential. Thus, if a cell model is touched by a visitor, the educator should provide necessary information about the function and organization of this cell. The use of materials like those produced in the museum collaborates on effective inclusive programs in non-formal educational settings, as these materials can be manipulated by all people, bringing greater similarity in attending to different publics. In conclusion, the production and use of tactile didactic models are importantto teach microbiology, since they facilitated the understanding of concepts about structures that cannot be seen with the naked eye in a multisensory way.

Supported by Fundação Butantan

10. Others

10.01 TstKMK, a new member from the scorpion β-KTx subfamily: identification through venom gland transcriptomics and predicted structure characterization.

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Introduction: *Tityus stigmurus* is a widely distributed scorpion species in Northeastern Brazil, known to cause severe human envenomations. In this work we used a transcriptomic approach to have an insight into the molecular repertoire of the non-stimulated venom glands of *Tityus stigmurus* scorpion. **Objectives:** Structurally characterize, by bioinformatic tools, one of the new potassium channel toxins found in the transcriptome. **Methods:** The cDNA library was constructed, 540 clones were sequenced and grouped into 37 contigs (more than one EST) and 116 singlets. Toxin homology modeling was performed with the MODELLER 9.10v suite, Phyre2 Web Server and I-TASSER. Molecular dynamics simulations were performed using the GROMACS Simulation package. Model structures of the venom peptide were submitted to the Cluspro server to be docked to Kv1.2 protein structure (PDB code: 3LUT). **Results and Discussion**: Forty-one percent of ESTs belong to recognized toxin-coding sequences, being transcripts encoding antimicrobial toxins (AMP-like) the most abundant, followed by alfa KTx-like, beta KTx-like, beta NaTx-like and alfa NaTx-like. Our analysis indicated that 34% of the transcripts encode "other possible venom molecules" and 15% of ESTs are similar to cellular transcripts. One of the significantly representative toxin found was TSTI0003C (TstKMK), it has a cDNA sequence of 538 bp codifying a mature protein with 47 amino acid residues, corresponding to 5,299 Da. The three-dimensional structure of this potassium channel toxin from the *T. stigmurus* scorpion was obtained by computational modeling and refined by molecular dynamic simulations. Furthermore, we have made docking simulations using a Shaker Kv-1.2 potassium channel from rats as receptor model and proposed which amino acid residues and interactions could be involved in its blockade. The transcriptomic profile of *Tityus stigmurus* resting venom glands is mainly composed of antimicrobial peptides, ion channel toxins and anionic peptides. Of the ion channel toxins, β-KTx subfamily is poorly characterized, though it is very representative in some scorpion venoms. The predicted structural features of the TstKMK toxin resemble that of other β-KTx. However, its molecular docking to Kv1.2 potassium channels showed some striking differences from the well-known α-KTx subfamily.

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10.02 Evaluation of 2 ways for measuring changes on the number of adipocytes *in vivo*

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Introduction: The differentiation of mesenchymal stem cells in preadipocytes into the stroma of adipose tissue is well-known *in vitro*, but the occurrence of this adipogenesis *in vivo* is controversial due to the difficulty in monitoring this event. Lipocrite (Li) (fraction of the total volume occupied by isolated adipocytes in suspension), mass of whole adipose tissue (MAT), the mean diameter (Dm) and the mean volume (V) of adipocyte, as well as the inferred density of the lipid drop as 0.91 $g/10^{12} \mu m^3$ (Sports Med., 11:277,1991) and inferred mass of adipocyte (MAC) are parameters which association indirectly could provide approximate values of the number of adipocytes (NA) and thus could help to evidence this phenomenon *in vivo*. Furthermore, the food deprivation induces an increase in the lipolytic rate, which could alter NA. **Objectives:**This study aimed to evaluate whether the calculations of Li/V and MAT/MAC can show the occurrence of altered NA *in vivo* after fasting. **Methods:**Male Wistar rats, 90 days old, 350-377 g, submitted to normal feeding (C) or to food deprivation for 72 h (FD) have 3g of their retroperitoneal fat pad removed. The adipocytes were then isolated from these fat pad by digestion and differential centrifugation. In the resultant suspension Li was measured by microcapillary centrifugation at 400 g, and Dm (in µm) was measured by morphometry under light microscopy using the software Image Pro-Plus® 4.0. Subsequently, the following calculations were proceeded: $V(mL)=\pi Dm^3/6$, $MAC(g)=0.91xV$, and NA, by (i) $(Li/V)x10^{12}$ and by (ii) MAT/MAC. The experimental procedures were in accordance with the protocol 684/09 approved by the Ethics Committee on Animal Use of the Instituto Butantan. Data were expressed as mean±SEM and analyzed statistically (FD versus C) by unpaired two-tailed Student's t test ($p < 0.05$) using GraphPad Prism™ software. **Results and Discussion:**As results, Li: FD* (0.18±0.010), C (0.06±0.004); Dm: FD* (77.7±2.00), C (107.1±0.74); NA, by (i): FD* ($7.48\pm0.52x10^6$), C ($0.95\pm0.05x10^6$) and by (ii): FD* ($13.74\pm1.13x10^6$), C ($5.13\pm0.100x10^6$). In conclusion, the increase of Li in FD is due to hyperplasia, since it is known that the increase of Li occurs through the increase of Dm (hypertrophy) and/or NA (hyperplasia) and FD had lower Dm and higher Li than C. This increased NA in FD was clearly demonstrated by the calculations using Li or MAT/MAC, both resulting in values at the same order of magnitude and with similar statistical differences between C and FD, although calculation by MAT/MAC provides higher NA and lower NA ratio between FD/C than the calculation by Li. Data suggest that the associated measurements of lipocrite and mean volume of adipocytes provide reliable estimation of the number of adipocytes in suspension. In the particular case of fasting, the change in the number of adipocytes, calculated by lipocrite or by the ratio between mass of whole adipose tissue/inferred mass of adipocyte, suggests the occurrence of adipogenesis, which is probably a homeostatic response to lipolysis during food deprivation and an adjustment to provide an increased energy storage when there is a new food supply.

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10.03 Accessibility of information on the zoological collections of the IBU

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Introduction: The Butantan zoological collections are known for their great value to science in Brazil and worldwide, because of their specificity in animals of medical and veterinary importance. In 2011, the Butantan Institute received the financial support from Governo do Estado de SP, to promote the construction of a new building where the collections would be stored, and the new Special Laboratory of Zoological Collections for their management. With the approval of the INFRA Project (09/54921-4) by FAPESP it was possible the computerization of the collections and the creation of a specialized software, named Sophia Acervo, to make available the information of all material stored at the collections. The Butantan Fundation made possible the hiring of trained human resources to computerize and operate the information at the new software, as well as to work at the collections reorganization, especially those who have been burned by fire occurred in May 2010. **Objectives**: This project aims to enter and organize the collections data, which are gathered in index-cards and collection-books, into a computer database, and make them available online. The project will also contemplate the remounting of damaged slides; standardization and implementation of barcode labels; scanning of all type species images; development of the Collections Standard Operating Procedure, which will control data organization. **Methods:** The collections computerization is a process comprised by stages. At first, all data of the 5 collections contained in index-cards and collection-books were entered into a Microsoft Excel program that was used as the basis to another computer program, which can be applied on the internet, allowing real time access. **Results and Discussion**: All the collections are already computerized. Up to day, these data are being checked and migrated to the Sophia Acervo software. Once this phase is completed, complete information will be available on the internet to public access. It is already possible to access the Acari and Opiliones collections at the LECZ site on the web. The next to be made available will be the Chilopoda collection. The specimens of Arachnida and Reptiles Collections are in the initial conference since many were lost during the fire. Thus, these two collections are the latest available and have migrated to the Sophia Acervo.

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10.04 Standartization and evalution of equine influenza vaccine

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Introduction: The currently circulating influenza A (H3N8) viruses were first isolated in 1963, when a group of thoroughbred horses exhibiting sings of respiratory disease arrived in Miami by air from Argentina. The causative agent was a previously unknown influenza virus, now as influenza A (H3N8) virus. Subsequently, equine influenza A (H3N8) virus has caused episodic outbreaks in horse populations throughout the word, leading to substantial disruption to and economic losses for equestrian industries as described by Lewis et al. 2011. The hemagglutinin (HA) protein is of key importance in the control of equine influenza because HA is the primary target of the protective immune response and the main component of currently licensed influenza vaccines. The bivalent influenza vaccine with two influenza A(H7N7) and A(H3N8) subtypes has been widely used in many countries that have high in reducing this disease in immunized horses. **Objectives:** Standardize and evaluate the equine influenza vaccine efficiency according to the manual it OIE/2008. **Methods:** To preparation of the bivalent vaccine against equine influenza were used two sub-type of the influenza virus A(H7N7) and A(H3N8). These two sub-types were replicated in MDCK cultured cells. After that were inactivated with formaldehyde (1:2000). The virus antigens were evaluated for hemagglutinating activity and safety testing in laboratory animals. Immunization of ten horses followed protocols of the manual, received two vaccinations at intervals 21 and 42 days. Serological tests of hemagglutination inihibition (HI) and the Single Radial Hemolysis (SRH) were used to measured the antibody responses of horses in the sera collected before and after vaccination. **Results and Discussion:** It was found that the experimental bivalent vaccine against equine influenza induced increase the immune response in vaccinated horses. The antibody levels, either by the techniques of HI and HRS, detected in their sera 42 days after vaccination were 3 fold of the antibody levels before vaccination of these animals. The results obtained can be considered that the vaccine was efficient immunization against equine influenza.

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10.05 Molecular and serological characterization of Influenza A isolated from wild birds in the state of São Paulo, Brazil

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Introduction: Avian Influenza virus belongs to Orthomyxoviridae family. The last years several low pathogenic avian influenza subtypes have caused outbreaks and epidemic in human and poultry. The wild and migrating birds may be participating of maintenance and interspecies transmission of the 16 subtypes of the Hemagglutinin and 9 Neuraminidase in nature. **Objectives:** Our study aimed subtyping samples positive by serological test haemagglutination inhibition (HI) technique and Molecular Biology.**Methods:** The samples from species *Elaenia mesoleuca* (2), *Sporophila lineola* (1) *Sporophila caerulescens* (1), *Vireo olivaceus* (3), *Columbina talpacoti* (3), *Paroaria dominicana* (2), were collected in reserves and experimental field stations located in the São Paulo State - Brazil, during the years 1997 and 1998. The samples were identified by HI test (according WHO) using the 20 antibody patterns anti-influenza A type and one for the influenza type B and RT-PCR and Sequence analysis of Hemaglutinina and Neuraminidase genes. **Results and Discussion:** The HI test demonstrated that 12 samples presented an antigenic close relationship with A/HongKong/1/68 (H3N2), A/ Equine/Miami /63 (H3N8) and A/Duck/ Ukraine/ 63 (H3N8) antiserum. The sequencing analyses of Hemaglutinin and Neuraminidase genes of these 12 isolates revealed a high homology with H3N2. Phylogenetic analysis and genetic variability compared with GenBank sequences representing several countries have shown that our samples showed a close homology with the virus subtypes Siena (1991) Victoria (1990) and Beijim (1989). Amino acid analysis indicated that there are non-synonymous mutations in the gene of Hemagglutinin (Y153F, K172A, D175H, T264I) and Neuramidase (T55S), exclusive of our samples compared to samples of other countries. Our samples when analyzed NA protein, showed no mutations in amino acids E119V and N274Y that conferring resistance to inhibitors Neuraminidase subtype N2.

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10.06 Effect of BjKgn (Bothrops jararaca Kininogen) on processing proteases of pro-inflammatory cytokines and on biological activities of macrophage

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Introduction: A protein named BjKgn, isolated from *B.jararaca* has similarity to the human high molecular weight kininogen (HK), since it has a molecular mass of 110 kDa, releases a pharmacologically active peptide and is an inhibitor of cysteine-protease. Both BjKgn and HK exhibit inhibitory activity on Jararhagin (JAR). JAR is capable of inducing the release of tumor necrosis factor-alpha (TNF-α) which is expressed on the cell surface and cleaved by a metalloprotease, called TACE (TNF-α converting enzyme) resulting in the release of soluble TNF-α. Another important cytokine that participates during the poisoning by Bothrops snakes is the Interleukin-1β (IL-1β). It is intracellularly processed by ICE (Interleukin converting enzyme), a cysteine-proteinase which belongs to the family of caspases. **Objectives:** The objectives of this study were to evaluate the possible inhibitory effect of BjKgn on ICE, TACE, and the effect on some functions of peritoneal adherent cells, such as spreading, phagocytosis, production of hydrogen peroxide (H_2O_2), nitric oxide (NO) and TNF-α and IL-1β. **Methods**:The tests with TACE and ICE were performed using fluorescent substrates corresponding to their respective enzymes and different concentrations of BjKgn. Spreading and phagocytosis tests were conducted using adherent peritoneal cells stimulated or not, and different concentrations of BjKgn (0.5 µg, 1µg, 1.5 µg, 2 µg and 3µg). Tests for production of H_2O_2, NO and cytokine release were performed only with stimulated cells and 3 µg of BjKgn. Cells were adhered and incubated in the presence or absence of BjKgn for 1 hour in all *ex vivo* experiments but the ones for the cytokine release, which the cells were remained on contact with the protein for 12 hours. **Results and Discussion**: The results showed that BjKgn, on the used doses, was able to competitively inhibit TACE and ICE enzymes with the inhibition constants of 2 mM and 370 mM, respectively. Regarding the activities of peritoneal exudate cells, the protein did not affect any of the activities at doses of BjKgn used, since the cells presented the ability of spreading, phagocytosis, production of H_2O_2 and NO similar to those displayed by cells of the control group. Despite ICE and TACE inhibition *in vitro*, BjKgn did not influence on the release of TNF-α and IL-1β. We have concluded that BjKgn protein is an inhibitor of cysteine-proteases and metalloproteases since it inhibited TACE and ICE *in vitro* and that, at least at the doses used, the protein does not affect the metabolic or biological adherent peritoneal cells function and It was not able to inhibit release of TNF- α and IL-1β by these cells.

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10.07 Modeling and structure analysis of E6 BPV-1 recombinant protein

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Introduction: Papillomaviruses (PVs) have been described as infectious agents of the vertebrate species, including domestic animals and human. Bovine papilomavirus type 1 (BPV-1) is commonly associated with the rise of lesions in penis, teats and udder in cattle and sarcoid tumors in equines. Viral oncoprotein E6 has a central role as a carcinogen factor because it binds to p53, a major tumor suppressor protein, inducing its degradation. In contrast to the many studies regarding the biological functions of E6, there are only few about its structure and biophysics, due to difficulties in obtaining soluble proteins. A structural model may be generated *in silico* with a high degree of confidence based on homologous proteins, which could enable different protein analysis. **Objectives:** *In silico*prediction of the 3D structure of E6 BPV-1 recombinant protein from sequences produced in the laboratory. **Methods:** The E6-1 recombinant sequence was submitted to BLASTP program using the PDB database to determine the most appropriate mold. The following programs were used: MODELLER6v2 to build protein models; SPDBV for visualization of 3D structures, generation of molecular surfaces, electrostatic potentials and performing the overlap between template and the model; PROCHECK and VERIFY-3D for validation; PDBsum to generate the topology diagram; Consurf to analyze the conserved regions and to identify the cation-π bonds; JaMBW for antigenicity studies. **Results and Discussion:** The degree of identity between the recombinant sequence and template (*PDB codes* 3PY7) was 99%, showing strong similarity. Ten models were created. The Ramanchandran plots confirmed the excellent quality of the models, with the best percentage found for models 2 and 6, being 94.2% and 5.8% the percentage of residues in the most favored and additional allowed regions, respectively. No model presented problems of planarity for peptide bonds and all models had Factor-G values above -0.5. Only models 3, 7 and 10 showed angular distortions. Data suggest that ten models were sterically valid and model 2 was considered the most suitable for analysis. According to the antigenicity graph, E6-1 showed one peak near amino acids 90 and 100. CXXC domains, highly conserved and required for the binding were identified. Four pairs of amino acids ARG/TYR and one pair ARG/TRP were verified as potentially capable of performing cation-π interactions. Differences in electrostatic potential were observed in divergent amino acids (T25S and I52T), but without predicted structural changes.

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10.08 Phylogenetic analysis of Sphingomyelinase D and evolution of the presence of disulfide bridges

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Introduction: Sphingomyelinase D (SMase D) is a venom enzyme from *Loxosceles* and *Sicarius* spiders responsible for the manifestation of dermonecrosis, intravascular haemolysis and acute renal failure. This enzyme is represented by several isoforms and, according to the number of disulfide bridges, SMase D may be classified to Class-I (one bridge) or Class-II (two bridges). **Objectives**:In order to understand the evolutionary significance of this classification we investigate the SMase D by a phylogenetic approach. **Methods**: We analyzed 463 sequences representing isoforms from different genera: *Loxosceles*, *Sicarius*, *Ixodes*, *Rhipicephalus*, *Arcanobacterium* and *Corynebacterium*. The phylogenetic reconstruction was performed by Neighbor Joining, with bootstrap (10,000 replications), on p distances. All analyses were performed with MEGA5 software. **Results and Discussion:** After multiple alignment (MUSCLE implemented in MEGA5), the sequences were classified in Class-I or Class-II, according to the presence of cysteines in defined positions. We found (37/463; 8%) Class-I sequences and (418/463; 90%) Class-II sequences. Thebacterialsequences(8/463, 2%)are toodivergentandcould not be classifiedby this criterion, but were analyzed by phylogeny to root the tree. We found that only *L. laeta*, *L. gaucho* and *L. sp* 4 GJB-2008 were classified to Class-I, all the others were Class-II (except bacterial sequences). The tree shows that all Class-I sequences were grouped at same clade, except *L. gaucho*. Our results indicate that Class-I SMase D evolved recently and independently in *Loxosceles laeta* and in *L. gaucho*. Because these spider species are widely distributed and their venom are recognized as very toxic, we propose to test the hypothesis that Class-I SMases are responsible for such higher toxicity, as the presence of disulfide bridges can alter substantially the flexibility of the polipeptide.

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10.09 Study of the relationship between weight and age in Guinea Pigs

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Introduction: At the Butantan Institute the animals (*Cavia porcellus*) produced by the Central Vivarium are destined for the toxicity and imunobiological potency tests. Differently from other lab rodents, they present a long gestation period (63 days in average) and their pups are considered precocious since they are born already well developed with full body hair, open eyes, and they move within few hours after their birth, ingesting solid food after three days. This colony requires specific space and handling in order to lessen the difficulties brought by the natural biology of the species. The users of these animals make their requests based on the weight factor. Therefore, the production vivariums must be attentive to the significant alterations to the weight x age ratio, since such alteration may indicate changes in the phenotype profile of the colony and interfere with the protocols established by the users. **Objectives:** This study has as its objective the presentation of the weight x ratio regarding the animal colonies of the Butantan Institute. **Methods:** The data was obtained at the department of production of guinea pigs of the Central Vivarium at the Butantan Institute. Fifteen (15) guinea-pigs (*Cavia porcellus*) were observed, within a control sanitary environment and were kept within a standard environment during a period that began with the animals' birth until they reach adulthood. The animals had *ad libitum* access to water and food. Weekly weightings were performed, always at the same day and hour, resulting in data concerning the weight x age ratio for both sexes. **Results and Discussion:** The animals used for this study were born with an average weight of 101.4 grams. They gained an average 53.9 grams per week, which made possible to conduct the weaning when the animals were two weeks old and with an average weight of 204.4 grams. The weaning process is extremely difficult at any age, being considered as a stressful time for the pup due to the break of the mother/pup link. However, with the intend of preserving the matrixes and reducing the pre-weaning mortality caused by inter-species competitiveness within the same environment aggravated by the maturity of the pups, we performed a precocious weaning (at 14 days of age) as an alternative to maximize the production of animals. We have observed that despite their young age, the animals that were weaned precociously presented a good performance, with a satisfactory weight gain, reaching 250 grams at 21 days of age. Such is the required weight for the toxicity and imunobiological potency tests. However, the weight of the animals should not be the only factor to be considered for their use as biological raw materials. It is of the utmost importance to consider the physiological maturity of the animal, taking into account the weight x age ratio as a safer parameter, one that will avoid repetition, becoming a tool of reduction, and therefore more ethical.

10.10 Employment of the potassium dichromate in the control chart establishment to *Biomphalaria glabrata*embryos (Mollusca: Gastropoda)

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Introduction: Ecotoxicological assays allow assessing the toxicity of chemicals when taken in environmental representative species, contributing to the identification of pollution sources and helping in the detection and estimation of deleterious effects on living organisms. Control charts are used to establish an acceptable range of test variability, accuracy and reliability of the results. Assays should be conducted regularly using reference substances, that can be organic or inorganics compounds used in standard procedures, which provide information to the interpretation of the results from the toxicity tests. Environmental protection agencies recommend some reference toxicants, as potassium dichromate ($K_2Cr_2O_7$). **Objectives**: In this work, assays with embryos of the freshwater snail *Biomphalaria glabrata*, intermediate host of schistosomiasis, were performed to prepare the control chart required in toxicity assays applied in the molluscicide screening and ecotoxicological studies. **Methods**: Embryos were exposed to potassium dichromate for 24 and 48 hours to determine EC_{50} (concentration that affected 50% of the exposed organisms to the test substance). Mortality and malformation were the endpoint. Six assays were performed. **Results and Discussion**: The mean values of EC_{50}forblastulae, gastrulae, trochophore and veliger stages, exposed for 24 h were 20.89mg/L, 17.53mg/L, 11.78mg/L e 5.90mg/L,to embryos exposed for 48 h, EC_{50} values were 13.18mg/L, 11.97mg/L, 6.19mg/L and 2.10mg/L, respectively. The results remained within the confidence limits in all assays and indicated that sensitivity of organisms reared in laboratory is under control. Potassium dichromate was both embryolethal and teratogenic in all embryonic stages. The hexavalent chromium salt is highly soluble and can be easily absorbed, passing the cells membrane and crossing the shell surface and the body of the embryos. The mechanisms by which chromium salts exert teratogenic action may be attributed to the inhibition of mitosis by interaction with genetic material.This occurs during the reduction of hexavalent chromium to trivalent chromium, where highly reactive radical species are released, which is capable of causing DNA damage. With these assays, it waspossible to establish the potassium dichromate concentrations and the control chart to be used in *Biomphalaria* tests.

10.11 Hemostasis in *Crotalus durissus terrificus* snakes: the role of thrombocytes

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Introduction: Most reptiles, including snakes, present high levels of circulating anticoagulants and the absence or deficiency of some coagulation factors. There is evidence of effective extrinsic and intrinsic coagulation pathway in *Crotalus durissus terrificus*(*C.d.t.*) blood. However, the contribution of circulating thrombocytes in the mechanism of hemostasis of this specie is not known.**Objectives:** The aim of this study was to investigate the role of thrombocytes in hemostasis of*C.d.t.* **Methods:**Blood was collected by puncture of aorta of five adult snakes anesthetized with sodium thiopental (CEUAIB nº 788/11). Whole blood clotting time and plasmatic fibrinogen (colorimetric method) were measured. Thrombocytes, erythrocytes and leukocytes count was performed in Neubauer improved chamber.Morphological characterization by transmission (Zeiss LEO 906E) and scanning electron microscopy (FEI Quanta 250) were performed using routine techniques.The thrombocyte function was assessed in aggregometer Chronolog in whole blood with collagen (2.5 μg/mL) and bovine thrombin (0.1 up to 8.8 U/mL), and thrombocytes rich plasma with calcium ionophore (20 mM) and ADP (4.05 mM).The adhesiveness of thrombocyte was assayed incone and plate(let) analyzer ImpactR.**Results and Discussion:**Clotting time was prolonged (127.5 ± 64 min), but the level of fibrinogen (2.13 ± 0.32 g/dL)was normal comparing with human. Circulating blood thrombocytes (14.4 ± 6.4 x 10^9/L) showed, in transmission electron microscopy, typical organelles such as α-granules, dense bodies and open channels system and when observed in scanning electron microscopy, showed up as elongated cells with smooth surface. Regarding the function, thrombocytes were activated by collagen (5.8 ± 2.6Ω), calcium ionophore (24 ±10.7%) and thrombin (results varied due to the presence of inhibitors). As described in other reptiles, aggregation was not observed with ADP. The adhesion observed (1.7 ± 0.7% with heparin and 1.35 ± 0.6% sodium citrate 3.2%) was lower than in humans (11%). Our results show that *C.d.t.* thrombocytes, by their morphological and physiological characteristics, play an important role in hemostasis with participation of the intrinsic pathway of coagulation.

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11. PIBIC/PIBITI Program

11.01 Study of the cytotoxicity and genotoxicity of potential antiviral substances extracted from tick eggs waxy secretions.

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Introduction: Previous studies demonstrated that an interesting antiviral activity is present in the waxy secretion involving tick eggs. As the control of antiviral infections is of great interest in public health, in this study we evaluate the cytotoxicity and genotoxicity of these waxy secretions. **Objectives:** The aim of this work is to find out if the waxy secretions from a few different ticks are cytotoxic or genotoxic to cell cultures. **Methods:** Ticks were grown in colonies maintained in the Parasitology Laboratory at Butantan Institute. The waxy secretion was obtained by washing the egg masses in ice-cold phosphate buffer pH 6.8, the resulting solution was dried and ressuspended to yield an appropriate final concentration, and the tests were performed in VERO cells. Cytotoxicity was evaluated by the MTT method and genotoxicity by the "comet" method. The waxes from the ticks *Rhipicephalus sanguineus,Rhipicephalus. (Boophilus) microplus* and *Amblyomma aureolatum* were evaluated and the results are very promising. **Results and Discussion:** In concentrations as low as 0.5 mg/mL all the samples tested caused no damage to the cells morphology as the crystal violet assay showed and the MTT tests confirmed. The best results were obtained with the eggs wax from *R. (Boophilus) microplus*. The fractionated wax from *A. cajennense* was toxic for the cells in concentrations below 0.5 mg/mL. In waxes presenting lower cytotoxicity effects genotoxicity tests are currently under evaluation through comet tests.

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11.02 *Ornithodoros brasiliensis* (Acari: Argasidae): transcriptome of salivary glands

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Introduction: *Ornithodoros brasiliensis* Aragão is an endemic tick to Brazil, restricted to highlands of the state of Rio Grande do Sul. It is a very aggressive species to humans, causing fever, great pain and intense inflammatory response at the bite site. **Objectives**: To build a cDNA library from the salivary glands of this tick. **Methods:** Pirosequencing was used, Model GS-454 Junior to sequencing the cDNA obtained from mRNA from the salivary glands, producing a total of 81,178 sequences (reads) with an average size of 400.8 bases. Were assembled 4,558 contigs using the program Genomics CLC-(CLCBio) and annotated by software Blast2GO, based on sequence similarity. These were compared to various banks (GenBank, GO, Enzyme Codes, InterPro, KEGG). **Results and Discussion:** The results showed that 20.93% of them were classified as belonging to the secretory pathway and 14.77% as proteins of membrane, confirming the tissue of salivary gland. The main families of transcribed genes were lipocalins (15.6%), moubatins which can be sub-classes of lipocalins (6.23%), protein with a tail acid (1.63%), and metalloproteases (1.23%). The sequences of interest present in the salivary glands were cloned into bacterial heterologous system. The recombinant proteins were expressed in sufficient quantities to analyze the sequences of lipocalins, moubatins, metalloproteases, cell cycle modulating and related to analgesia. All these proteins show functions that modify the coagulation activities, modulating the immune response and inhibition of platelet aggregation, among others, showing their potential for development of bioproducts with medical and industrial interests.

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11.03 Search for plasma proteins from *Crotalus durissus terrificus* snake responsible for protection against his own accidental poisoning and study of their potential use as antivenoms

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Introduction: Snake venoms are a complex mixture whose proportion and specific features vary among the different known species. Venom toxicity is due to the presence of proteins, and their lethal action is attributed mainly to neurotoxins. So, human accidents caused by snakes of the genus *Crotalus* are characterized by their myotoxic, neurotoxic and coagulant activities due to several proteins present in the venom, such as crotoxin, gyroxin, convulxin and crotamine, among others. However, some snakes are resistant to toxicity of the poison itself, by several mechanisms of neutralization. **Objective:** the present study aims to investigate plasma proteins from *Crotalus durissus terrificus* snake (C.d.t) responsible for protection against self-envenomation, assessing the role of these as antivenoms. **Methods:** plasma and venom proteins from C.d.t. were measured by absorbance at 280 nm using a plate reader (Epoch biotechnology). Both venom and plasma were submitted to 1 D and 2D SDS-PAGE (12% for venom and 10% for plasma). The gels were stained with Coomassie G. Gels were also subjected to western blotting using PVDF membranes (Hybond-GE Healthcare). PVDF membranes containing C.d.t plasma proteins were incubated with C.d.t venom and anti-crotalic serum was used to recognize venom proteins linked to snake plasma proteins. **Results and Discussion:** we have seen, by electrophoresis techniques, the protein profile of plasma and venom from C.d.t. The venom of this snake shows several bands, corresponding mainly to the following proteins, crotamine (4.8 kDa), crotoxin (23 kDa), gyroxin (35 kDa) and convulxin (68 kDa). The results obtained by the western blotting enabled us to identify proteins that were recognized by this same animal venom proteins, suggesting that the recognition of these proteins can have an antivenom activity. However, the negative control of this experiment also presented some bands that were recognized by the anti-crotalic serum, even without prior incubation with C.d.t. venom. Thus, these bands do not represent necessarily the plasma protein binding with proteins of the venom, being an inespecific reaction. In this way, the results of the 1D “western blotting”, presented some bands that were strongly recognized on the membrane incubated with the venom, while on the control membrane these bands were weakly developed and sometimes even appeared. This reaction suggests that the C.d.t plasma has proteins that bind effectively to venom proteins and can inhibits these venom proteins. Thus, after numerous attempts to obtain western blotting with a lesser degree of nonspecific reactions, we have seen some bands and spots that reacted specifically in the membranes previously incubated with C.d.t. venom. So, these bands and spots will be extracted for subsequent identification by mass spectrometry.

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11.04 Expression of proteins induced by 5-aminolevulinc acid in primary hepatocyte cell culture

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Introduction: In patients with Acute Intermittent Porphyria (AIP), there is accumulation of 5-aminolevulinic acid in liver, leading the cell internal balance to a pro-oxidant state. The maintenance of this state triggers cellular pathways, which may lead to development of hepatocellular carcinoma (HCC). The knowledge about this events enables the use of a few cell markers, as the proteins Bak, Bcl-2, the factors p53, Nfκβ and the enzymes catalase (CAT) and superoxide dismutase (SOD) as indicators of the pro or anti-oxidant state in the development of HCC in patients with AIP. **Objectives:** Elucidate the cellular mechanisms possibly involved in growth of hepatocellular carcinoma in patients of acute intermittent porphyria with use of oxidative cell markers. **Methods:** Treatment of hepatocytes primary cell cultures of Wistar lineage rats with 5-aminolevulinc acid in different concentrations and analysis of the cell-lysates with Western Blotting with radiation by intermittent microwaves. The protein concentration has been quantified by bicinchonic acid (BCA) spectrophotometry; the peroxidation state of lipids and the quantity of reduced glutathione has been also analysed. **Results and Discussion:** Three series of hepatocytes were extracted from different animals. The samples were quantified by BCA, lipid peroxidation state and dosage of reduced glutathione. The preliminary results indicate high concentration of cells given the high efficiency of the extraction, making necessary dilutions for further analysis. Among the next steps, the lysates will be tested through Western Blotting with radiation by intermittent microwaves. Furthermore, new tests on protein concentration, reduced glutathione and lipid peroxidation will be made. By monitoring these cell markers we hope clarify the intermediate cell states possibly involved in the development of hepatocellular carcinoma in symptomatic patients of AIP.

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11.05 *Ornithodoros mimon* (Acari: Argasidae): transcriptome of gut

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Introduction: The role of the argasid tick *O. mimon* as vector of pathogens is still unknown, but it is an aggressive species to human and lives in home environment. Because of this, the species is maintained in the laboratory for different studies. **Objectives:** Present work aims to build a cDNA library from the internal organs of *O. mimon,* such as the salivary glands and digestive tract. **Methods:** Sixty-one female of *O. mimon* were fed on rabbits and they were dissected into two post-feeding periods (24h and 48h) for the extraction of internal organs, such as intestine, salivary glands, ovary and skin. The samples of organs were placed in a solution that preserves the RNA and then placed in -80 ° C. Samples of gut and salivary glands were defrosted and processed by molecular techniques for the extraction and purification of mRNA, in order to obtain and to analyze cDNA. **Results and Discussion:** The amount of mRNA obtained from the salivary glands of *O. mimon* was insufficient for sequencing process. In contrast, the mRNA extracted and purified from the gut was suitable for sequencing the sample. The parcial results of the transcripts of the digestive tract of *O. mimon*, presented genes associated with energy metabolism, digestion and transport of elements, machinery replication, transcription and modulation of protein. The transcripts present in these organs will be selected for the production of recombinant proteins, which will be used in biological activities studies such as toxins, anesthetics and anticoagulant factors to enables applications in the pharmaceutical industry.

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11.06 Evaluation of the expression of the recombinant human Granulocyte-Colony Stimulating Factor (rhG-CSF) in *Brevibacillus choshinensis*, by using different growing conditions

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Introduction: The proliferation, differentiation, maturation and activation of hematopoietic blood cells are under the control of growth stimulating molecules, known as Colony Stimulating Factor (CSF). These growth factors are lineage-specific, controling the maturation and development of unique lineages. Within this family, the Granulocyte-Colony Stimulating Factor (G-CSF) is the molecule related to the polymorphonuclear granulocytes lineage, mainly to neutrophils. Related to its activity, this molecule is widely used as a biopharmaceutical, in the treatment of neupenia, a condition characterized by a low number of circulating neutrophils, with is a common condition in cancer and AIDS patients. **Objectives:** We evaluated the possibility of G-CSF production in the cell culture supernatant using the expression system based on the bacteria *Brevibacillus choshinensis* – which has a remarkable capacity for protein secretion – by varying the growing conditions. **Methods:** We evaluated the level of recombinant protein expression by changing the culturing conditions. Cells were cultured in medium with different composition, using several volumes of culturing media and with different antibiotic concentration. For each growing condition, the expression of the recombinant protein was determined through Western blot. **Results and Discussion:** Bacteria grown in different media showed different capacity to produce the recombinant protein: the expression was detected in bacteria grown in TM media, although it was not observed in 2SY media. On the other hand, it was shown that the cultivation in small volumes of media (5 ml) led the bacteria to secrete the recombinant protein, while the cultivation in higher volumes (250 ml) induced the bacteria to retain the expressed recombinant protein inside the cell. When the antibiotic concentration was increased, it was not detected a regular pattern of the recombinant protein expression: protein expression was detected for culturing bacteria in some antibiotic concentrations, but it was absent for other ones. We concluded that the culturing conditions are associated with the recombinant protein expression, although these associations were not always clearly evident, especially in the case of variation in antibiotics concentration, suggesting that other unevaluated factors may be also influencing the recombinant protein expression.

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11.07 Characterization of anti-EspB antibodies for application in capture assay

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Introduction: Diarrheagenic *Escherichia coli*is responsible for 30-40% of the diarrhea cases in developing countries. Enteropathogenic *E. coli* (EPEC) is a category of diarrheagenic *E. coli* responsible for a large number of cases of acute and persistent diarrhea in several countries. Enterohemorrhagic *E. coli* (EHEC) can cause hemorrhagic colitis and syndrome hemolytic uremic. EPEC and EHEC induce attaching and effacing (A/E) lesions on intestinal enterocytes, characterized by intimate bacterial adhesion, destruction of microvilli, and accumulation of polymerized actin in pedestals beneath intimately attached bacteria. The A/E lesion formation is due to the presence of a type III secretion (T3S) system that injects effector proteins into the host cell. Among these proteins, EspB and EspD, are translocated to the host membrane where they form a pore structure, thus excellent target for diagnosis, because the initial diagnosis is essential for minimize the sequelae caused by both pathogens and immunodiagnosis represents a rapid and simple alternative. **Objectives:** Preparation and characterization of hybridomas producing anti-EspB antibodies from clone 4B4. **Methods:** Anti-EspB hybridoma4B4 clone, previously obtained in our laboratory, was submitted to limiting dilution. K1 clones were tested by ELISA in order to define their immunoglobulin's isotypes, their reactivity to EspB, as well the ability to capture the antigen with polyclonal antibodies. **Results and Discussion:** The hybridomas produced immunoglobulin's type IgG1, IgG2a, IgG2b and IgG3. The monoclonal antibodies were reactive and did not compete to polyclonal antibodies by EspB protein. Due to the displayed isotype purity two clones were selected, IgG1 and IgG2b-producing antibodies. Thus, the obtained antibodies have ideal characteristics for their use in a rapid test, conferring sensitivity and specificity to a capture assay, which has a great importance as diagnosis method for neglected disease, such as diarrhea.

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11.08 Snakebites by *Bothrops jararaca* in the state of São Paulo

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Introduction: In Brazil there are around 20.000 snakebites per year, and it's a major public health problem. The genus *Bothrops* is responsible for about 90% of all cases of snakebite, especially the species *Bothrops jararaca*, responsible for 93% among the *Bothrops*. **Objectives:** The aim of this study was characterize the epidemiology of accidents caused by *Bothrops jararaca* and evaluate possible influences of biological variables found in specimens that have caused accidents in São Paulo. **Methods:** A survey of the number of species in the period from 01-25-2010 to 03-03-2013 was conduced for São Paulo. The book number 19 of the animals records from the Vital Brazil collection in Instituto Butantan was used for obtain data relating of snakebites for characterize a possible profile of the *Bothrops jararaca* accidents. **Results and Discussion:** The analysis of the data reveals that the species *Bothrops jararaca* is the most responsible for the snakebite of the genus *Bothrops* in São Paulo. Records of 238 snakes that causing the accidents, 186 were causing by *Bothrops jararaca*. The city where higher accident rate was the metropolitan region of São Paulo, with 67 accidents. Data also analyzed of the other cities of the São Paulo state shows that the Embu city is responsible for 15 accidents, Cotia 13, São Bernardo do Campo 11, San Roque 9, Mairipora 7 and Embu das Artes 6 accidents. Results showed that 84 of the accidents were caused by young females while 39 were males. In adults, females were responsible for 25 accidents, while in males were 19, therefore the results indicate that both young and adults, there was a predominance of females causing snakebites. The anatomical area of the patients most affected were the feet, hands and fingers, which can be related to agricultural activity in field and human action, providing the meeting of these animals to human. The results of the identification of stomach contents of *Bothrops jararaca* showed that 28 were fed, 91 not fed and 65 not identified, so in general, these activities may be related to foraging, thermoregulation and especially the reproductive period of snakes. The foraging activity and most intense period of activity are factors that can increase the snakebites.

Supported by PIBIC

11.09 Effect of platelet releasate on inflammatory pain: Study of the action mechanism

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Introduction: Recently, our group demonstrated that platelet depletion inhibits hyperalgesia induced by carrageenan and *Bothrops jararaca* snake venom in rats. In addition, intranplantar (i.pl.) injection of whole platelets or platelet releasate (PR), equivalent to $200x10^9$ platelets/L, evoked mechanical hyperalgesia, suggesting that platelets are essential to the genesis of inflammatory pain. **Objectives:**Herein, we evaluated the possible mechanisms involved in PR-induced hyperalgesia. **Methods:** Hyperalgesia was evaluated in male Wistar rats submitted to the paw pressure test after i.pl. injection of PR (100 µL), in the concentration equivalent to $200x10^9$ platelets/L, and pain threshold was assessed after 1, 2 and 4 h (CEUAIB 848/11). The same concentration was used to evaluate edema. Mediation of the hyperalgesic response induced by PR was investigated by pretreating animals with anti-rat platelet antibody (1.25mg/kg, i.v), fucoidan (selectin inhibitor, 5mg/kg, i.v.), methysergide (antagonist of serotonin receptors, 5 mg/kg, i.p.) or indomethacin (COX inhibitor, 100 ng/pata). **Results and Discussion:** The hyperalgesic response induced by PR lasted 4 h, and its edematogenic activity was of low intensity and short duration (30 min), suggesting that hyperalgesia was not associated with edema. Circulating platelets were important for PR-induced hyperalgesia, since systemic depletion of blood platelets inhibited this effect. Moreover, fucoidan blocked PR-induced hyperalgesia, suggesting that either selectins or neutrophils participated in PR-evoked hyperalgesia. However, histological analyses of hind paws of rats injected with PR showed that in periods when the hyperalgesic effect occurred, leukocyte infiltration, especially of neutrophils, was mild. These findings demonstrated that neutrophils are not crucial for PR-induced hyperalgesia. Anti-platelet rat antibody failed to inhibit the hyperalgesic effect of PGE_2, an agent which causes hyperalgesia independently of inflammatory response, suggesting that platelets are involved in the genesis of inflammatory pain, but not in nociceptor sensitization. Furthermore, methisergide did not interfere in PR-induced hyperalgesia, suggesting that serotonin receptors are not involved in this effect. On the other hand, indomethacin inhibited PR-inducedhyeralgesia, indicating that COX and its metabolites are essential for the hyperalgesic activity of PR. These data suggest that platelets trigger inflammatory pain, and that neutrophils are not essential for this effect. In addition, COX metabolites are involved in PR-induced hyperalgesia.

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11.10 Effects of streptozotocin–induced diabetes on muscarinic acethylcholine receptors in the rat hippocampus

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Introduction: Metabolic syndrome is close related to diabetes mellitus type 2, obesity and neurodegenerative processes, being a serious public health problem. In turn, muscarinic acethylcholine receptors (mAChRs)in the hippocampusparticipatedecisively in the modulationof cognitive functions, learning and memory, which are compromisedin neurodegenerative processes. Diabetes-induced muscarinic M_1 receptor downregulation was reportedpreviously in the hypothalamus, brainstem and pancreatic islets of streptozotocin (STZ)-induced diabetic rats. At present, the effects of hyperglycemia on the expression of mAChRs have not been explored in the hippocampus. **Objectives:** The present investigation reports the effect of STZ–induced diabetes on the expression and affinity of these receptors. **Methods:** To induce diabetes, STZ diluted in citrate buffer pH 4.5 was intraperitoneally (ip) administered (50 mg/kg BM) between 3:00 and 4:00 h of light period to weaned 21-25 days old male Wistar rats that had been fasted for 18 h. The normal control group received only citrate buffer. After 30 h, STZ-injected animals with blood glucose levels higher than 200 mg/dL (STZ-diabetic), and those injected only with citrate buffer with blood glucose levels between 60 and 90 mg/dL (control), were selected. In saturation-binding experiments, hippocampus membranes, obtained from control and STZ-diabetic rats at 140 days of age, were incubated with $[^3H]$QNB (0.05-8.0 nM) in the absence and presence of atropine (1 μM) (30°C/1h). **Results and Discussion:** Scatchard analysis of specific binding yielded a dissociation constant (K_D) of 0.48 ± 0.11 (n=3) and 0.11 ± 0.01 nM (n=3), respectively, for control and STZ-diabetic. The binding capacity (B_{max}) obtained was, respectively, 987.23 ± 99.48 (n=3) and 165.50 ± 88.05 fmol/mg protein (n=3) for control and STZ-diabetic. The results indicate that the STZ-induced diabetes induced a significant decrease on the expression and increase on the affinity of mAChRs in the rat hippocampus (P< 0.05, Student t-test). These actions of diabetes on mAChRs in hippocampus might be a key step mediating cellular events important for learning and memory.

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11.11 Biochemical characterization of immunoglobulin G and IgM from plasma of Magellanic penguins

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Introduction: Global population ofMagellanic penguins, *Spheniscus magellanicus*, is approximately 1.3 million pairs breeding in Argentina, Chile and the Falkland Islands. These birds visit the Brazilian coast during migration in the South Atlantic Ocean during winter. Due to oil pollution, overfishing, and climate variation, they may be affected by infectious diseases, such as avian malaria. The immune system accounts for detecting and eliminating distinct pathogens by different mechanisms mediated by cells or soluble components, such as immunoglobulins (antibodies). **Objectives:**Considering the relevance of these animals as sentinels of marine environment, and the fact that theirimmunobiology is poorly understood,our aim was to characterize the biochemical properties of IgG and IgM antibodies from the plasma of penguins in order to contribute to the development of serological tests for avian malaria. **Methods:**Plasma samples from clinically healthy penguins provided by Santos Municipal Aquarium were delipidated and precipitated with caprylic acid associated with ammonium sulphate. Precipitated proteins were purified using size exclusion chromatography (Sephacryl S-200 column). Efficiency of purification steps was analyzed by SDS-PAGE, ELISA and Western blotting assays. The protein content was characterized by two-dimensional gel electrophoresis (2DGE) and analyzed by Image Master 2D 7.0 software.Purified immunoglobulins were evaluated for their binding affinity to *Canavalia ensiformis, Sambucus nigra, Datura stramonium* and *Artocarpus integrifolia* lectinsusing an enzymatic assay (ELISA). **Results and Discussion:** 2D-PAGE analyses of purified IgM showed 3 and 6 spots corresponding to heavy and light chains, respectively; heavy chains exhibited relative molecular mass around 86 kDa, and isoeletric point (pI) ranging from 3.4 to 5.08, while the light chains had 27 kDa and pI around 5.27- 6.76. Purified IgG showed 6 and 4 spots, corresponding, respectively, to heavy and light chain;. heavy chains had molecular mass of 60 kDa and pI ranging 6.8-8.2, while the light chain showed 26 kDa and pI 5.2 - 6.8, similar to IgM light chains. These results are similar to those obtained for other heavy and light chains from bird immunoglobulins. Binding assays with distinct lectins revealed that IgM and IgG present *N*-linked oligosaccharides with fucose, sialic acid and *N*-acetylglucosamine residues, and absence of O-glycans. Furthermore, these results indicate that this N-linked glycan chain is of complex-type, as observed for IgG from chicken and mammalians.

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11.12 Seasonal variation of body temperature in male and female *Crotalus durissus* and *Bothrops jararaca* under semi-extensive captive conditions throughout autumn

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Introduction: The temperature is the main determinant factor for distribution and diversity patterns of ectothermic animals. However, information about body temperature and thermal ecology of Neotropical snakes are still very scarce. Depth studies on thermal ecology and its relationship to the life history of snakes are critical to the maintenance in captivity and conservation actions. **Objectives:** The aim of this study was to obtain the thermoregulatory profile of *C. durissus* and *B. jararaca* under semi-extensive captive conditions, as well as evaluate variations in body temperature during the breeding season (autumn) of males and females. **Methods:** Data of the body temperature (T°*Co*) and substrate temperature (T°*Su*) of *Crotalus durissus* and *Bothrops jararaca* were collected twice a day (morning and afternoon) with an infrared thermometer (Instrutherm IT - 870 High temperature). The environmental temperature (T°*Am*) was applied to the meteorological station of IAG/USP, and the data were analyzed using the BioEstat (version 5.3). **Results and Discussion**: *Crotalus durissus* had the greatest variation in mean T°*Co* (20.5 °C for males and 22.4 °C for females) in relation of*Bothrops jararaca* (11.4 °C for males and 10.3°C for females F = 114.5574 p = <0.001). This variation may be related to conditions of captive enclosure of *C. durissus*, which does not accurately simulate the original habitat conditions (humidity, temperature, rainfall characteristic of the Cerrado and Caatinga). Being located in the São Paulo city where the climate is tropical/subtropical humid, *Crotalus durissus* probably require adjustments to physiological and behavioral thermoregulation as observed during autumn, when *C. durissus* kept his T°*Co* higher than *B. jararaca.* Pearson correlation analysis (p <0.05) showed that the environment temperature as the temperature of the substrate have a strong influence on the thermoregulation processes in ectotherms due to their dependence on external heat.

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11.13 Cloning and expression of recombinant insularin (INS) in free form

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Introduction: Disintegrins represent a family of cysteine-rich, low molecular weight proteins occurring in venoms of various vipers. They bind with a high affinity to numerous integrins and are potent inhibitors of platelet aggregation and cell adhesion. Insularin (INS) is a disintegrin from *Bothrops insularis* venom that was subcloned and expressed on a prokaryotic expression system as a fusion protein with glutathione S-transferase (GST). Della-Casa et al. (2011) demonstrated the ability of the recombinant disintegrin insularin fused to GST (GST-INS) to inhibit both platelet aggregation induced by ADP and HUVEC adhesion to fibrinogen. However, in this work, the cleavage and removal of the GST tag-protein was not successfully achieved. For this reason, all the biological tests were performed with the protein in fused form (GST-INS). However; the obtention of recombinant insularin in free form may enhance its biological activity. Therefore, the aim of this project is to obtain recombinant insularin in fusion to a small ubiquitin-related modifier (SUMO), and then remove SUMO by specific protease called ULP1.The protein SUMO has advantage over GST because its cleavage is based on its tertiary structure recognized by ULP1, avoiding allosteric impediment cleavage. **Objectives:** Obtention of insularin in in free form (INS). **Methods:** The construction of pGEX-INS (produced by previous studies) was treated with restriction enzymes Bam*HI* e Hind*III* to extract the insert INS. This insert was then used for ligation into pSMT3 vector, which contain SUMO sequence, generating the construction pSMT3-INS. This construction was then submitted to sequencing and used to transform into chemically competent cells *E. coli* C43 (DE3). The expression of insularin fused to SUMO (SUMO-INS) was performed for 4 hours at 37 °C by adding 1 mM of IPTG. The cells were lysed by sonication and the recombinant protein was purified by affinity chromatography. The purified recombinant protein was then dialyzed in phosphate buffer saline (PBS) and analyzed by 15% SDS-PAGE.**Results and Discussion:** Expression of insularin in fusion with SUMO protein was successfully achieved in *E. coli* C43(DE3) using pSMT3 vector. After purification, SUMO-INS showed to be soluble and exhibited an expected molecular mass around 22 kDa on SDS-PAGE. Further experiments are being conducted in order to remove SUMO from insularin by ULP1 protease.

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11.14 Contribution of cytosolic calcium to the vasoconstriction response induced by angiotensin II in the aorta of the rattlesnake *Crotalus durissus terrificus*

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Introduction: Angiotensin II (Ang II) produces its vascular effects in mammalian species through the interaction with angiotensin receptors, AT_1 and AT_2. Previously we have demonstrated the presence of a functional Ang II receptor in the aorta of two Brasilian snakes, pit viper and rattlesnake, which has a low affinity for the selective AT_1 and AT_2 antagonists. Besides, this Ang II receptor produces its effect by a mechanism independent of phopholipase C activation. **Objectives:** The aim of this study was evaluate the role of the cytosolic calcium in the vasoconstriction response induced by Ang II in the aorta of the rattlesnake. **Methods:** Functional assay was used to obtain cumulative concentration-effect curves to Ang II (10^{-10} – 10^{-6}M) in the absence (control) and in the presence of the following substances: SKF96365 (store-operated calcium channel [SOC] inhibitor), caffeine (ryanodine receptor [RyR] agonist) and cyclopiazonic acid ([CPA] sarcoplasmatic reticulum Ca^{2+}-ATPase pump inhibitor) in aortic rings of *Crotalus durissus terrificus*. The importance of extracellular calcium to contractile response induced by Ang II was analyzed by punctual (10^{-7} M) concentration of the peptide in Ca^{2+}- free physiological solution plus 1mM EGTA, and also by cumulative concentration-effect curve to Ang II obtained after removal extracellular Ca^{2+} and caffeine addition. **Results and Discussion**: SKF96365 ($3x10^{-5}$M, n= 7) and caffeine (10^{-3} and $2x10^{-3}$ M, n= 3-8) were able to reduce the Ang II maximum effect, respectively, in 40%, 53% and 68%. CPA (10^{-3} and $3x10^{-3}$ M, n= 4-5) did not modify the Ang II concentration-effect curve. Depletion of the extracellular calcium strongly inhibited (71%) Ang II (10^{-7}M, n= 5) – induced contraction, and a recover occurred after restory the normal calcium concentration in the medium. Extracellular calcium removal plus caffeine addition (n= 3) completely abolished the Ang II cumulative concentration curve. Together these data support the participation of extacellular calcium in the contractile process induced by Ang II, while SOC and RyR participate of this mechanism. Moreover, our results indicate that the calcium–ATPase pump from the sarcoplasmatic reticulum is not involved in the Ang II response. These results contribute to the knowledge of the signal transducer mechanism of the Ang II receptor in vertebrates.

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11.15 Reporting of parasitism in humans by *Ornithodoros fonsecai* (Acari: Argasidae) in the municipality of Nobres,in the state of Mato Grosso, Brazil.

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Introduction: In Brazil the studies with argasid ticks increased in the last 10 years resulting in the description of six new species. The *Ornithodoros* genus is represented by 15 species, and four of them are endemic to this country. *Ornithodoros fonsecai* was recorded only in Brazil, associated with bats in caves of the municipalities of Bonito, state of Mato Grosso do Sul and Nobres, state of Mato Grosso, respectively. Its biology was held in 2012, using a colony of ticks from Bonito. There are reports of tick bites in humans to *O. fonsecai* from Mato Grosso do Sul. **Objectives:** Record the parasitism of *O. fonsecai* in humans in a cave of the municipality of Nobres, state of Mato Grosso, and the maintenance in laboratory of the specimens collected in the same locality. **Methods:** In December 2011, specimens of *O. fonsecai* were collected in a cave known as "Gruta Lagoa Azul", located at 80 Km from Nobres municipality, state of Mato Grosso. Ticks were sent to Instituto Butantan, and after identification they were deposited at the IBSP Acari Collection. In June 2012, a new collection was performed in the same cave and different stages of live specimens were collected. Ticks were brought to the laboratory and kept under controlled temperature and humidity. The identification of the species was carried out through morphological and molecular studies. **Results and Discussion:** Ticks collected in 2012 were initially kept in a BOD with temperature at 27° C ± 1° C. However nearly all nymphs and adults died. Ticks that survived were fed on rabbits and some of them changed for males and females. After feeding, the adults mated, and about a month later, one of the females laid eggs. In an attempt to approach the cave environment, the temperature of the BOD was increased to 30° C ± 1° C. The specimens collected in Nobres seem to differ of those from Bonito in their biological cycle. However, this only can be confirmed when the biological studies of the ticks from Mato Grosso, still in progress, is completed. Unlike of the reported in the literature, people parasitized by *O. fonsecai* of Nobres felt no pain or discomfort at the time of the bite and even the disappearance of the edema. After a few minutes of parasitism, the tick bites resulted in an intense inflammatory response, with redness and swellings of moderate circumference, which persisted for more than a week. It would require a comparative study between the ticks of these two different locations to find out if there is any difference in their saliva components. The project to analyze the saliva components of the ticks collected in Nobres, Mato Grosso state, has already started.

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11.16 The contribution of TLR4 and TLR2 in the synthesis of IL-10 during the activation of M□DM with *Bordetella pertussis* and *Bordetella parapertussis*

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Introduction: *Bordetella pertussis* e *Bordetella parapertussis* are human pathogens and etiological agents of the whooping cough disease that affects the human respiratory system. The Toll-like receptors (TLRs) are expressed in macrophages and are important in the innate immunity, with the adaptor molecule MyD88 being essential for its activation and cytokine production. Many aspects concerning the contribution of these receptors in the development of immune responses and the control of infections are still unknown. The presence of IL-12 is crucial during the innate response for the development and control against these pathogens. This cytokine can be produced by activated macrophages and the mechanism that controls its production during an infection by *Bordetella spp* is not yet clear. Previous data shows that macrophages activated by *Bordetella,* produced significant levels of IL-12p40, which is controlled by the presence of IL-10. We investigated this possibility and the contribution of TLRs in this mechanism. **Objectives:** Analyze the participation of IL-10 in the control of IL-12p40 synthesis and the contribution of TLR4 and TLR2 during macrophage activation by *Bordetella pertussis* and *Bordetella parapertussis*. **Methods:** The M□DM were obtained from the femur and tibia of C57BL/6 mice, MyD88 $^{-/-}$, TLR2 $^{-/-}$ and C3H/HeJ (hyporesponsive for LPS). The cells were stimulated with 30µg/ml of soluble antigen *B. pertussis orB. parapertussis*. LPS of *E.coli* (2 µg/ml) or Zymosan (1000µg/ml) were used as controls in the experiments. The supernatant was collected after 24 hours for IL-12p40 and IL-10 dosage. **Results and Discussion:** Our results demonstrated that macrophages from C57BL/6 produced significant levels of IL-12p40 and IL-10, in response to the stimulation with antigens from both bacteria. The production of IL-10 was much higher than IL-12p40. The synthesis of IL-10 involves Toll-like receptors and the molecule MyD88, with drastic reduction of this cytokine during macrophage activation of *knockout* mice in MyD88. The synthesis of IL-10 involved the participation of signals regulated either by TLR4, as well as TLR2. The participation of TLR4 was more accentuated, and the reduction of IL-10 was most significant. The production of this cytokine was always higher during cell activation with *B. parapertussis* antigen. These data show TLR4 and TLR2 participate in the regulation of the synthesis of IL-10 during the first contact of these bacteria with phagocytes, although there are structural differences in LPS and the absence of pertussis toxin in *B. parapertussis.*

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11.17 G protein-coupled estrogen receptor (Gper) mediates rapid activation of phospholipase c pathway in rat hippocampus

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Introduction: 17β-estradiol (E2) induces structural and functional effects on hippocampus of adult female rodents. Female rats in late proestrus have 30% higher density at apical dendritic spines, and 32% more synapses than rats in estrus. Furthermore, E2 is involved in learning and memory. In fact, genomic and non-genomic (rapid) actions of E2 are observed in hippocampus via the classical nuclear estrogen receptors, ESR1 (ERα) and ESR2 (ERβ). In addition to the activation of these receptors, the membrane-initiated E2 signaling mechanisms are also observed by activation of GPER (G protein-coupled estrogen receptor). These rapid actions play a role in neuronal excitability, signal transduction, cell death, neurotransmitter release and gene expression in the hippocampus. **Objective**:The role of GPER on rapid responses in hippocampus function is unclear.Thus, the aim of this study was to investigate the effects of GPER-selective agonist G-1 on intracellular signaling pathway phospholipase C (PLC)-phosphoinositide hydrolysis in the hippocampus obtained from rats in different phases of the estrous cycle. We now report the effects of G-1 on PLC-mediated phosphoinositide hydrolysis in hippocampus obtained from rats in estrus.**Methods:** Hippocampi obtained from rats in estrus (approved by the Research Ethical Committee from Instituto Butantan, nº 373/07) were incubated in the absence and presence of G-1 (1 nM). The total [^{3}H]-inositol phosphate accumulation was measured, as previously described. **Results and Discussion:** G-1 (1 nM) induced a rapid time-dependent increase of total [^{3}H]-inositol phosphate accumulation in hippocampus. The maximum effect was observed at 1 min ($51.24 \pm 4.91\%$ above basal levels, n=8). The total [^{3}H]-inositol phosphate accumulation induced by 1-min treatment with G-1 (1 nM) was blocked by pretreatment with selective GPER antagonist G-15 (1 nM) for 30 min, but not by ESR1 and ESR2 antagonist ICI 182,780 (1 nM, 30 min).The total [^{3}H]-inositol phosphate accumulation induced by 1-min treatment with G-1 (1 nM) was also blocked by pre-treatment of the hippocampuswith U73122 (PLC inhibitor), suggesting that the GPER are upstream component that regulate total [^{3}H]-inositol phosphate in this rapid action.These results indicate that GPER induces phosphoinositide hydrolysis mediated by PLC in rat hippocampus. This rapid action in hippocampus might be a key step mediating cellular events important for learning and memory.

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11.18 LgRec1-EGFP, a new chimeric protein as new tool to track phospholipase D from *Loxosceles gaucho* venom

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Introduction: The *Loxosceles* genus is recognized as a public health problem in Brazil. Patients bitten by this spider generally manifest marked local inflammatory reaction and dermonecrosis. Among several toxins in the venom, phospholipases D (PLDs), also called dermonecrotic toxins are the most studied because it has been shown to be responsible for the main effects observed in loxoscelism. This toxin family is able to induce dermonecrosis, platelet aggregation, renal disorders, local inflammation and hemolysis. Despite presenting various biological effects, its mechanism of action or targeting to cellular receptors are not known. Currently, to study the target of several proteins, they are being fused to green fluorescent protein (GFP), originally from jellyfish *Aequorea victoria*, which function as a reporter protein. **Objectives:** Create a hybrid molecule composed of a fosfolipase D (LgRec1) from *Loxosceles gaucho* and EGFP to evaluate its function as a molecular marker that could track LgRec1 without the need of a chemical fluorophore. **Methods:** The cDNA encoding the recombinant protein LgRec1 was cloned into vector pAZ in frame with EGFP sequence. This construction was submitted to sequencing and denominated EGFP-LgRec1 and used to transform BL21(DE3)pLyz cells to achieve expression. The recombinant chimera was purified from the supernatant by immobilized metal affinity chromatography (IMAC) and analyzed by SDS-PAGE. Sphingomyelinase assay was performed using Amplex® Red Sphingomyelinase Assay Kit (Life Technologies). Platelet rich plasma (PRP) aggregation assay was monitored in a Chrono-log aggregometer, with temperature maintained constant at 37ºC. **Results and Discussion:** EGFP-LgRec1 was successfully expressed in soluble form growing *E. coli* BL21(DE3)pLys at 30ºC. SDS-PAGE showed a band of molecular size around 60 kDa as expected. The hybrid molecule showed sphingomyelinase and platelet aggregation activities, indicating that the fusion of EGFP to LgRec1 did not interfere with the main activities of this toxin. Further experiments to analyze interaction of this hybrid molecule with cells are being conducted. The fusion of LgRec1 to EGFP may contribute to known the cellular targets of this toxin, which will help to understand its mechanism on the pathophysiology of Loxosceles envenomation.

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11.19 Evaluation of cytokine levels in brain of adults rats injected with TsTX-I toxin isolated from *Tityus serrulatus* scorpion venom.

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Introduction: The scorpionism is considered a health problem in Brazil and the *Tityus* genusisresponsible for most of the accidents with humans in this country. The scorpion venom acts on autonomic centers releasing mediators like adrenaline, noradrenaline and acetylcholine. Moreover, inflammatory mediators are also released after scorpion bites. It has already been observed that TsTX-I toxin isolated from *T. serrulatus* scorpion venom causes electrographic and behavioral alterations and in some cases these alterations are associated with variation in the level of some cytokines. **Objective:** The objective of this study is the evaluation of the intracerebral level of cytokines in rats after the intrahippocampal injection of the isolated toxin TsTX-I. **Methods:** The rats (240-260g) were divided in three groups, with five animals each one. These animals were submitted to a stereotactic surgery for implantation of cannulaguides. Four days after the surgery the groups of animals received an intrahippocampal injection of 0.125μg/μL of TsTX-I toxin, 1 μL of saline (negative control group) or 1.5 μg/μL of kainic acid (positive control group), respectively. Four hours after the injection, the animals were sacrificed and the brain tissues were removed and processed to dose cytokine levels. **Results and Discussion:** It was observed an increase in the level of IL-6 in the hippocampus and of IFN-γin the hippocampus and brain after the toxin injection. However, it was observed a decrease in IL-1αlevel in hippocampus and in IL-1β and IL-10 levels in brain. This fact could may be explained by a rebound effect in which an increase of cytokine levels is followed by a decrease. The results obtained show that the TsTX-I toxin isolated from *Tityus serrulatus* scorpions venom changes the level of some cytokines in the nervous system.

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11.20 Study of the anti-proliferative and pro-apoptotic effect of metalloproteinase-disintegrin jararhaginin human pancreatic tumor cells

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Introduction: Metalloproteinases present in snake venoms have shown potential anti-metastatic models of carcinogenesis. The jararhagin is a metalloprotease isolated from the venom of *Bothrops jararaca*, which acts as a potential anti-cancer agent by competing for receptors of the tumor cell. **Objectives:** Evaluated the potential anti-proliferative and pro-apoptotic of jararhagin, in culture cells of adenocarcinoma and carcinoma human pancreatic. **Methods:** Cell viability was assessed by MTT assay for determination of IC50%. Lipid peroxidation was determined to assess oxidative membrane damage and determined by flow cytometry expression markers involved in cell cycle pathway p53, p21 and p27, cell death caspase 3 active, Bcl-2 and BAX and TNF-αDR4. **Results and Discussion:** The cells Mia Paca-2 and BX-PC3 treated with different concentrations of jararhagin demonstrated a significant difference of sensitivity, being the Mia Paca-2 more sensitivewith IC50% equal to 0.001 nM and BX PC3 with IC50% equal 0.0119 mM. There were reduction of cell adhesion in both cell lines and formation of multicellular aggregates that increased when exposed to concentration higher than 1nM. The treatment of Mia Paca-2 cells resulted in a gradual increase lipid peroxide formation, in the lowest concentrations that 0.00001 mM. BX-PC3 cells produce more lipid peroxides, at concentrations of 0.01, 0.001, 0.0001 mM. This was found to be dose-dependent effects, showing a positive correlation between toxin concentration and malondialdehyde production. The Mia Paca-2 cells increased the level of expression of BAX and decreased expression of Bcl-2, however, no significant changes in expression levels of active caspase-3 active, TNF-DR4, p53, p21 and p27. The BX-PC3 cells decreased the level of expression of Bcl-2 protein and increase the expression of Bax protein and caspase 3 active, showing a trend in modulate for apoptosis, however, no significant changes in the expression of TNF-DR4, p53, p21 and p27.The half maximal inhibitory concentrationIC50%obtained in this work demonstrated that the jararhagin showed cytotoxic effects between the human pancreatic tumor cells. There were changes in the morphological and kinetics of cell growth and loss of the tumor cell adhesion induced by jararhagin, there was also an increase in production of free radicals. The expressions of the markers showed that the jararhagin increased expression of the pro-apoptotic Bax protein in both cell lines and caspase 3 active in BX PC-3. It has been also shown that jararhagin reduce Bcl2 expression. The jararhagin is a potent inhibitor of tumor growth.

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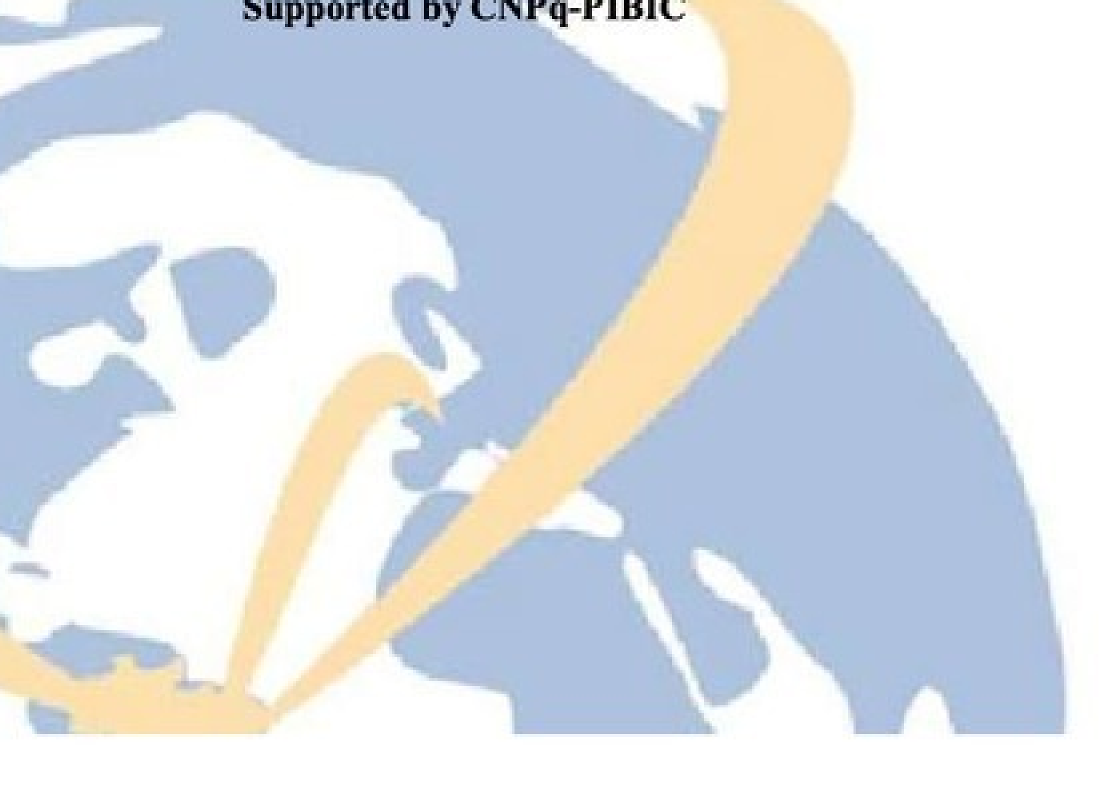

11.21 Identification of the binding site for LexA2 in *Leptospira interrogans* serovar Copenhageni

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Introduction: *Leptospira interrogans* can survive in water for weeks before infecting the next host, being exposed to a wide spectrum of DNA damaging agents. The occurrence of DNA damage can block replication fork generating regions of single-stranded DNA, which in turn activates a system called SOS. Little is known about the operation of this system in Leptospira. However, a study made in our laboratory with *L. interrogans* serovar Copenhageni revealed the presence of two *lexA* genes: one is common to all leptospires (*lexA1*), while the other is unique to this serovar and probably acquired by horizontal transfer (*lexA2*). Furthermore, the concentration of mRNA for the genes *lexA1, lexA2, dinP, recA* and *recN* increased after exposition to UV-C. LexA1 is capable of binding to their promoters, suggesting that the increase in their expression is due to releasing of the repressor from the SOS box. **Objectives:** The objective was to determine the binding sequence and possible targets of the LexA2 repressor using a series of Electrophoretic Mobility Shift Assays (EMSA). **Methods:** The EMSA was performed using lexA2up (promoter region) amplified by PCR and labeled with a terminal DIG as probe. Binding reactions were carried with purified recombinant LexA2. Competition assays used non-labeled probes, added to the binding reaction after the labeled one, in 200 fold excess. **Results and Discussion:** We first tested the binding of LexA2 to the promoters of the genes controlled by LexA1 using competition assays. LexA2 was only capable of binding to its own promoter region. New competition assays were performed with oligonucleotides corresponding to two palindromes found in this sequence (ATTCN$_{13}$GAATand TTGTAN$_{10}$TACAA), with three or nine flanking nucleotides. The ATTCN$_{13}$GAATsequence had no effect, while the TTGTAN$_{10}$TACAA one was able to disrupt the lexA2up-LexA2 complex. However, the competition was more effective with the sequence containing the largest number of flanking nucleotides, most probably as a consequence of stronger structural stability of the interaction protein/oligonucleotide. Thus, the DNA sequence recognized by LexA2 in the promoter region is probably TTGTAN$_{10}$TACAA.

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11.22 Search for new peptidic substrates for metallo and serine peptidases present in the venom of *Bothrops jararaca*(BjV):inhibition of this activity by the commercial antivenom

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Introduction: Snake venom poisoning is a public health issue for many countries. Studies show that the number of accidentssurpasses the number of fatalities from several other tropical diseases. In addition, snake bites only joined the list of neglected tropical diseases recently, in April 2009, showing that it was not seen as an important public health issue by WHO. In Brazil, *Bothrops* spp poisoning is responsible for 90% of snake bites and*Bothrop'*svenoms are rich sources for proteolytic enzymes (65% of the composition). The treatment recommended for bothropic poisoning is the application of the bothropic antivenom which inhibits most of this arsenal of proteases, but not all. Most studies have demonstrated that BjV act on macromolecular substrates causing an imbalance of the prey's hemostatic system. There are only a few studies about the effects of snake venoms upon small molecules, like peptides **Objectives:** Search for new bioactive peptidic substrates for the metallo- and serine peptidases present in the BjV that could be related with envenomation symptoms, and check the inhibition of the activity by the commercial antivenom from Butantan Institute. **Methods:** The BjV was incubated with Met-enkephalin, Leu-enkephalin, insulin beta chain and Substance P bioactive peptides using the site-direct inhibitors PMSF (5mM), EDTA (100mM) and the bothropic antivenom from Butantan Institute. The hydrolysis of these peptides was detected by HPLC analysis. **Results and Discussion:** Initially we observed the hydrolysisof theinsulin beta chainby BjV as a positive control, as it has been described that bothserine- and metallopeptidasesare involved in this particular hydrolysis. We were able to determine that, in this case, metallopeptidases(76.4%) are more active than serinepeptidases (63.5%). When the antivenom was tested, only the hydrolysis by metallopeptidases was abolished, showing no serinepeptidases neutralization. Substance Pwasalso hydrolyzed by BjV, and this activitywas totally inhibited byEDTA, demonstrating the role of metallopeptidaseson the cleavage of substance P. The inhibition testswith thecommercialantivenomshowed total neutralization of thesemetallopeptidases, as no hydrolysiswasobserved. BothMet- andLeu-Enkephalin were nothydrolyzedby BjV, and these results comply with the lack of neural symptoms observed in the victims. Taken together, the results here presented show that metallopeptidases are well neutralized by the commercial antivenom, but not the serinepeptidases that hydrolyzed the insulin beta chain.

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11.23 Study of buffer variation in the purification of prothrombin complex in ANX Sepharose FF from human plasma using pseudo-affinity chromatography

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Introduction: The development of neutralizing inhibitors is the most challenging complication associated with the treatment of Hemophilia and use of bypassing agents like activated recombinant coagulation factor VII and activated prothrombin complex are the recommended treatments. Prothrombin complex proteins undergo a conformational change induced by Ca^{2+}, that also modifies the affinity to ion exchange resins. **Objectives:** The aim of the study is to compare three different buffers in the purification of prothrombin complex by anion exchange chromatography coupled with pseudo-affinity chromatography. **Methods:** Plasma is applied to the anion exchange column ANX Sepharose FF and the proteins that do not bind to the column are washed with the equilibrium buffer. An intermediate wash is performed increasing the concentration of NaCl to 200mM. The elution of the prothrombin complex is carried out with the 200 mM NaCl buffer with a linear gradient of calcium. It was tested three different buffers, citrate, Bis-Tris and MES. Chromatography fractions were analyzed by the activity of Protein C using the chromogenic method. The protein content was measured by the Bradford method and the protein profile by SDS-PAGE. **Results and Discussion:** In previous experiments, it was observed that the prothrombin complex could be eluted using pseudo-affinity chromatography, using citrate buffer 25 mM. In this study, it was tested two other buffers, Bis-Tris and MES, which were chosen because they do not form complex with calcium and have suitable pKa for the pH used in the experiments. The chromatogram of the purification using citrate buffer showed several peaks, while the two others buffers presented only one peak. The SDS-PAGE gels showed the protein profile of the fractions collected in the citrate purification is different from the profile of the fractions collected in the Bis-Tris and MES chromatographies, while Bis-Tris and MES fractions were quite similar. Gels of all 3 experiments showed that other proteins, with higher molecular masses than that of the prothrombin complex proteins, which have molecular masses between 60 and 70 kDa, also eluted with the increasing of calcium concentration.

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11.24 In vitro activity of piplartine analogs on *Schistosoma mansoni*

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Introduction: Treatment and control of the flatworm disease, schistosomiasis, rely on a single drug, praziquantel. The identification of new active compounds has been strongly recommended given the potential for drug resistance. Previously, in bioprospection studies with vegetal species, we have identified an amide derived from *Piper tuberculatum*. Piplartine was highly active against adult and young *Schistosoma mansoni* worms with no cytotoxicity for cultured mammalian cells. The molecular target of the compound is not established, as well as the mechanism of schistosomicidal action. **Objectives:** The aim of this study is to establish a model of structure-activity relationship of piplartine in *Schistosoma mansoni* through the analysis of synthetic analogues. **Methods:** Piplartine analogs were pre-dissolved in 3% DMSO before dilution in RPMI medium. Five worm pairs were exposed to 50 and 100 μg/mL of each analog in 24-well culture plates and incubated for 120 hours. Positive control group was exposed to 3 μg/mL praziquantel and negative control to 0.003% DMSO. We assessed the motility of the schitosomas for 120 hours; motionless worms were removed and considered dead. **Results and Discussion:** Among 33 tested analogs, 7 exhibited activity at one or both levels with no difference in sensitivity between male and female worms. IC50 values are being determined for the active compounds.

Supported by CNPq

11.25 Enquiry of popular treatments and therapeutic itineraries used in cases of accidents caused by venomous animals

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Introduction: The anthropological approach of health contributes to a better understanding about the relationship between physical and social environment in which cultural factors influence preventive and the therapeutic practices performed by traditional (dwelling and rural) communities. Traditional practices of cure are transmitted orally from one generation to the other. It is important for health professionals to learn how traditional people design their own itinerary in order to obtain relief of symptoms and cure of diseases. **Objectives:** To analyze how some dwelling communities, Belterra/PA, and rural groups, Juquitiba/SP, perceive venomous animals in their environment, the relationship they establish with these animals and how do people deal with accidents. **Methods:** Workshops and semi-structured interviews were carried out in those locations in order to identify the meanings attributed to venomous animals as well as how the therapeutic itinerary is organized. We also wondered to know what the relationship between traditional and institutionalized knowledge is. Workshops were performed in groups, gathering people as much as the leader of the community could ask to attend to. There those who were mentioned as references in solving disease problems were interviewed. After that, a chart was constructed to describe the different practices used in cases of accidents. **Results and Discussion:** 11 workshop and 17 interviews were transcripted in a total of 41 hours of interaction between traditional communities and researchers (including meetings with the local leaderships). From this material we perceived that traditional treatments applied to accidents with venomous animals respected the same logic of the biomedical system - relief of symptoms and elimination of the causative agent. For the accomplishment of those practices and treatments the population mobilizes its network neighborhood turning to them for seeking remedies and looking for help to reach the medical treatment. Therefore the therapeutic itinerary is done collectively, mobilizing the community as a whole and revealing the social nature of disease. Thus, the popular and medical systems are not regarded as opposite by the populations studied but each one acts in a level of treatment, using a logic pre-determined by the therapeutic itinerary.

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11.26 Crotoxin inhibits migration of endothelial cells in the presence of a tumoral stimulus

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Introduction: Several studies, *in vivo* and *in vitro* have demonstrated antitumor activity of Crotoxin (CTX), the major toxin of *Crotalus durissus terrificus* venom. The formation of new blood vessels is the principal process of angiogenesis and involves adhesion, proliferation and migration of endothelial cells to reach remote targets. Angiogenesis play a key role in tumorigenesis and metastatic process and the assembly of endothelial cells into new capillary tubes contributes to tumor development. **Objectives:** In the present study investigate the effect of CTX on the migration of the endothelial cells in presence of a tumoral stimulus. **Methods:** *Wound Healing Assay*: Endothelial cells (EC) were plated in a 24 well plate and after became confluent a wound was made with a sterile tip. Cells were washed with PBS and incubated with CTX (1.2 μg/mL) for 1 hour or only in presence of the RPMI 1640 medium (control). After 1 h, cells were washed again with PBS and incubated in the presence of RPMI 1640 containing 10% SFB (control) or with conditioned medium (CM) of MCF-7 tumor cells for 24 hours. After this period photos were taken and migration was determined measure the area that cells migrate into the wound. *Transwell Assay*: This assay was evaluated using polycarbonate filter Transwell inserts (6.5 mm diameter) with 8μm pores. EC ($1x10^6$/mL) were incubated in RPMI without serum (control) or in medium without serum, containing CTX (1.2 μg/ml) for 1 hour, at 37°C and 5% CO_2. After this period, the cells were centrifuged and ressuspended in fresh RPMI 1640 medium without serum. Then, 200 μl of this cell suspension containing $1x10^5$ were added on in the upper chamber of transwell insert precoated with collagen I. Inserts were placed in a 24-well plates and added 600 μl of RPMI 2% (control) or the same volume of tumor cell MC in the lower chamber. After 5 hours of incubation at 37°C and 5% CO_2, cells migrated to the lower side of the transwell inserts were fixed with methanol for 10 min and stained with Giemsa dye. After PBS washing to remove excess Giemsa dye, cells on lower side of the insert membrane in five random fields per insert were counted. **Results and Discussion:** The results showed that CTX decreased migration of endothelial cells ability in *wound healing* assay (RPMI: 52% and conditioned medium: 81%) and in *traswell assay* (RPMI: 22% and tumor cell MC: 37%). Taken together, the results and the pictures indicate that CTX inhibits the EC in migration, especially when incubated with conditioned medium, which may contribute significantly to the inhibitory action of the toxin on tumor growth.

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11.27 Detection and genetic characterization of symbiont bacteria *Wolbachia* in Culicidae

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Introduction: Mosquitoes of the family Culicidae are epidemiologically relevant owing to their vectorial capacity for several human/animal pathogens. The urban park "Parque Ecológico do Tietê" (PET) is unique epidemiological scenario in São Paulo city (Brazil): Owing to the fact that the park harbours dozens of mosquito species, the thousands of leisure tourists that visit the park weekly are under risk of infection by insect-borne pathogens. **Objectives:** In an attempt to enrich the basic knowledge on culicids from PET and to help the development of new strategies to control those mosquitoes, we investigated the presence of *Wolbachia* in those mosquitoes. The bacteria *Wolbachia* are vertically-transmitted obligatory endosymbionts of arthropods and nematodes. Owing to its capability of altering the reproduction and other physiological features of its hosts, *Wolbachia* has been proposed as a "biological tool" to control Culicidae vetors. **Methods:** We detected the presence of *Wolbachia* using the amplification and sequencing of the bacterial genes: *wsp* and 16S rDNA. **Results and Discussion:** Seven species (73% of the 216 individual mosquitoes) were infected by *Wolbachia*, including some abundant species of *Aedes* and *Culex*. Phylogenetic tree of the *Wolbachia* we found (based on 16S) was not congruent to the phylogeny of its respective culicid hosts, revealing that bacteria and hosts have not followed the same evolutionary history. In addition, the high similarity among 16S sequences lead us to hypothesize that *Wolbachia* transmission in the culicid community of PET is primarily horizontal. If correctly interpreted, this pattern may be suitable for paratransgenesis to reach several species simultaneously.

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11.28 To evaluate the action condroprotective in vitro and rLopap rLosac molecules in inflammatory models induced by IL-1

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Introduction: osteoarthritic diseases are chronic degenerative diseases that affect the joints in general. Produced in the process are pro-inflammatory mediators and cartilage degeneration occurs. Patients feel much discomfort and pain. The treatment is usually performed in order to reduce the inflammatory process relieve pain through analgesics, anti-inflammatory and / or cartilage protective effect of slowing the process of joint degeneration. Lopap, members of hemocalins, and Losac, a member of the lipocalins, two molecules are widely studied by the group led by Dr. Ana Marisa Chudzinski Tavassi, which have the potential cytoprotective especially under stress conditions. These molecules were produced in recombinant form by the group and called rLopap and rLosac. **Objective:** To evaluate the effects of rLopap and rLosac in cultured human chondrocytes, as the ability to express extracellular matrix molecules and induce cytoprotective. **Methods:** Chondrocytes were isolated from human patients with osteoarthritis and provided by the collaborating group of Albert Einstein. To mimic the inflammatory process present in the disease, cell cultures were exposed to interleukin-1ß (IL- 1 - associated with joint degeneration). After 24 hours in this condition pro-inflammatory held treatment of cells with rLosac and rLopap and evaluated cell viability after 24, 48 and 72. The cell cycle of the treated cells was analyzed by flow cytometry. The presence differential extracellular matrix molecules (Hsp47, fibronectin and collagen type 1) was analyzed by Western blot. **Results and Discussion:** The results showed that rLosac rLopap and increase cell viability in normal cells and cells subjected to treatment with IL - 1ß, showing its ability cytoprotective in inflammatory conditions. Furthermore, it was observed greater production of extracellular matrix molecules in the treated cells. Possibly, this effect in the case of rLosac, is related to the ability of this molecule to increase the cell metabolism. Corroborating this hypothesis is verified that the chondrocytes after treatment are in the G0/G1 phase, where, despite cells are quiescent, have high synthesis organelles, proteins, enzymes and RNA possibly reflecting the expression of matrix molecules. Thus, the results support the hypothesis that rLoasac and rLopap have cytoprotective capacity and may have potential as a prototype for the development of therapeutic agents for the treatment of osteoarticular pathologies.

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11.29 Chicken clotting system activation by *Bothrops jararaca* venom assayed by rotational thromboelastometry: an alternative *in vitro* micro-assay to monitor the efficacy of antivenom

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Introduction: In *Bothropsjararaca* accidents, metalloproteases such as factor X and prothrombin activators likely represent the most important protein class contributing to hemorrhage and are similarly toxic for both mammalian and avian species. Although being hemorrhagic *in vivo*, *B. jararaca* venom (BJV) presents procoagulant activity in *in vitro* assays. **Objectives:**The rotational thromboelastometric assay (ROTEM) was used to explore whether the chicken clotting system can be activated by this venom, in presence and absence of antibothropic serum (ABS). **Methods:** Clotting time (CT) values of recalcified (20 μL of 0.2 M $CaCl_2$) chicken platelet-poor plasma (cPPP) samples (290 μL) treated with 30 μL containing: (a) 0.9% NaCl (control); (b) crescent doses of BJV and (c) ellagic-acid (EA) based-reagent, giving a final volume of 340 μL (n = 6 per condition). **Results and Discussion:** The CT values of avian cPPP control samples were significantly prolonged (no clot for at least 30 minutes) when compared with that presented by rat platelet-poor plasma samples (from 2 to 4 minutes). BJV induced a dose-dependent procoagulant effect, significantly decreasing the CT values. At final concentration of 1.5 μg/mL, BJV decreased the CT values to 674±58 sec (p<0.001, ANOVA). Preliminary results showed that 0.28 μL of ABS neutralizes 2 μg of BJV, giving a good correlation with the effective dose 50% (ED_{50}) (1 mL neutralizes 6 mg of BJV). This dose of ABS did not prevent the decrease of CT of cPPP samples activated with EA-based reagent. Assessment of antivenom potency still relies on traditional mouse lethality assay (LD_{50}), but leading to animal suffering.The direct procoagulant effect of small doses of BJV and the efficacy of its antivenom (the ABS) to neutralize this effect can be quantified by the ROTEM assay in our conditions. This assay is being proposed as a novel quality control test for assessing efficacy of antivenom in in-process quality control tests. This assay incorporate the following characteristics: (a) a*utomation*; (b) *miniaturization and reduction* – quantification and standardization of the procoagulant activity of small doses of BJVand of ABS and *(c)* Low cost and preservation - local anaesthesia allows blood samples collection from adult chickens (at least 6 mL from each wing vein, allowing at least 20 ROTEM assays), without significant animal distress or necessity of euthanasia.

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11.30 The production of a new recombinant platelet aggregation inhibitor from *Haementeria depressa* leech

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Introduction: Hematophagous animals have developed a great variety of substances that help feeding and digestion of blood, keeping it fluid during these processes. Among the clones of the cDNA library from *H.depressa* leech salivary complex a new platelet aggregation inhibitor was previously identified, cloned in pPIC9K and expressed by *Pichia pastoris*. This recombinant inhibitor was able to inhibit platelet aggregation (WP and PRP) when the collagen was used as agonist. It was identified that the GPIb alpha receptor as the target of the new molecule. This compound was named Placolin and while interesting it should be further characterized for its biological functions. **Objectives:** The purpose of this work is to express and to purify the Placolin in bigger quantity to further continue its characterization. **Methods:**The clone was transformed in *Pichia pastoris* (GS115) by electroporation and expressed in BMGY/BMMY culture medium for 96 hours at 28°C with shaking 350rpm. The expression supernatant was dialysed against 3mM NaCl and submitted to gel filtration (Superdex 75). The profile of expression and the purification process were analyzed by SDS-PAGE. **Results and Discussion:** The expression process occurred satisfactorily with profile similar to the previous studies. The purification process was able to obtain a main band of about 21 kDa by SDS-PAGE as expected, representing the recombinant protein glycosylated. The protein yield of the process was 32 mg/L and the total of protein obtained was about 2 mg of protein. The amount of protein obtained will be used to give continuity to characterize this new inhibitor of platelet aggregation.

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11.31 Reproductive and physiological alterations caused by superinfection of *Wolbachia* on its host *Aedes albopictus*

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Introduction: *Wolbachia* infects arthropods and nematodes and is a maternally-inherited intracellular bacterium, which belongs to the group of alpha-proteobacteria. This endosymbiont has been detected in dozens of species of insects and its effects on the reproduction of their hosts have been increasingly studied. Due to these effects, *Wolbachia* is being used as a biological control of insects that are vectors of pathogens. Among the effects, which tend to be specific to each case of *Wolbachia*-host interactions, are: parthenogenesis, male-killing, feminization, cytoplasmic incompatibility and especially alterations in reproductive fitness. Our team detected *Wolbachia* in the mosquito *Aedes albopictus* collected in the city of São Paulo (Brazil). Remarkably, at least two *Wolbachia* strains (supergroups A and B) are present in that mosquito, an unusual fact in mosquitoes. *Ae. albopictus* is responsible for the transmission of pathogens that cause diseases such as dengue and yellow fever. Despite the relevance of the mosquito, the effects of *Wolbachia* infection on reproduction of this species has been poorly explored. **Objectives:** To establish two mosquito lab colonies: 1) naturally-infected by *Wolbachia*; 2) experimentally-disinfected (*Wolbachia* free). **Methods:** Four eggtraps were placed at urban park "Horto Instituto Butantan". From the collected eggs we obtained 150 adults. Some adults (10) were PCR-confirmed as to be infected by *Wolbachia*. The remaining individuals were presumably infected because infection by *Wolbachia* is generally ubiquitous. From the pooled offspring of all adults, two sets of 200 adults were haphazardly selected. One set was used to originate a colony naturally infected by *Wolbachia*. The other set was treated with tetracycline in order to eliminate *Wolbachia* infection. **Results and Discussion:** The *Wolbachia*-infected colony of *Ae. albopictus* was succesfully established. The tetracycline treatment partially eliminated *Wolbachia* infection (about 20%), as demonstrated by PCR tests. According to the literature, antibiotic treatment is usually needed to be carried out by two consecutive generations. The next step will be to treat the second generation to get a completely non-infected colony. Hitherto, no change regarding the mosquito reproductive aspects was empirically noted. The colonies *Ae. albopictus* will be compared concerning parameters of reproductive fitness, such as fecundity, fertility and longevity, with the scrutiny of statistical tests.

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11.32 Interaction of Crotamine with Giant Unilamellar Vesicles

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Introduction: Crotamine is a small basic myotoxin from *Crotalus durissus terrificus* rattlesnake venom with β-defensin scaffold. β-defensin is a family of cationic peptides with antibiotic activity involved in vertebrate innate immune system. Due to its cationic characteristic, those peptides have strong electrostatic interaction with bacterial membrane, which is negatively charged. Besides the myotoxic activity, crotamine has antimicrobial activity against some bacteria and fungi. **Objectives:** Our objective was to analyze the crotamine activity on giant unilamellar vesicles (GUVs), which mimics the phospholipid composition of bacterial and eukaryotic membranes. **Methods:** GUVs are ideal for qualitative inference due to their big size (10 - 100 μm), which allows observation by optical microscope. POPC,POPG and cholesterol were used to prepare three types of GUVs: only POPC, POPC:POPG 75:25 (mol%), and POPC:Chol 90:10 (mol%), the last two ones mimic prokaryotic and eukaryotic membranes respectively. Time evolution of GUVs morphology was followed by video microscopy right after they were incubated in glucose solutions containing from 1 to 50 μM crotamine. The behavior of each vesicle was observed by a phase contrast microscope at 63x magnification. To liposome rupture kinetics, it was used 10x magnification for observing a large number of vesicles. Membrane permeability alteration was observed by halo intensity variation along a single vesicle radial line using the software ImageJ 1.46. **Results and Discussion:** At doses greater than 5 μM, crotamine destabilized the lipid membrane (POPC:POPG) leading so fast to bilayer disruption that it was not possible to observe the rupture kinetics. Two micromolar crotamine destabilized 81% of POPC:POPG 75:25 GUVs (n = 571) during 1 h. However, in POPC and POPC:Chol 90:10 GUVs, crotamine activity was reduced to 57 and 45% (n = 598 and 1581, respectively). In all types of GUVs tested, crotamine burst vesicles without observation of any previous effect. On both GUVs (POPC:POPG and POPC:Chol), significant variation on halo intensity was not observed indicating that membrane permeabilization did not occur. On the other hand, 2 μM magainin I decreased the halo intensity of POPC GUVs due to the micropore formation. Magainin I is a well-known antimicrobial peptide from *Xenopus laevis* skin which permeabilizes vesicles. Concluding**,** crotamine has higher activity on bacterial-like than on eukaryotic-like membranes, disrupting lipid membrane without stable pore formation.

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11.33

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Introduction: Amblyomin-X is a recombinant protein obtained from the cDNA library constructed using salivary glands of the tick *Amblyomma cajennense*. It is a Kunitz type inhibitor, with potential therapeutic in inhibition of blood coagulation (FXa) and cytotoxicity of tumor cells. Initially, the cloning and expression of this gene were performed in bacteria *E. coli*. However, the protein contained a histidine tag (6 his) in structure, in order to have a better purification process. The "His Tag" is not agreed with the requirements of regulatory agencies for use in clinical trials, **Objective:** The aim of this study was to produce Amblyomin-X in *Pichia pastoris* system, without His tag, and obtain a pure protein with inhibitory activity over factor Xa and cytotoxicity, following concepts of Good Laboratory Practice in the production. **Methods:** Expression in yeast *Pichia pastoris* to obtain Amblyomin- X step purification of the recombinant molecule was performed with ion-exchange column Source-Q. Analysis of electrophoresis and Western blotting were performed to verify the presence of the protein. We also evaluated the inhibitory activity of FXa and cytotoxicity in fibroblast cells and SK-mel. Thus the system used for recombinant protein expression Amblyomin-X is made with a good yield and maintaining its activity. **Results and Discussion:** The protein yield after the entire process was 1.5 mg / L of fermentation. Although this quantity was unsatisfactory compared to the production of Amblyomin-X in *E.coli*, the protein was easily purified and the activities of inhibition of factor Xa and cytotoxicity in tumor cells were better. Moreover, the *P. pastoris* system has the advantage of direct expression in the culture medium, without the refolding step, performed in *E. coli* culture. A major breakthrough has been made in the study of this protein, particularly in relation to large scale production to obtain sufficient quantities to conduct pre-clinical trials, according to the requirements of regulatory agencies such as FDA and ANVISA.

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11.34 Gene expression induced by jararhagin-C, a disintegrin-type protein isolated from *Bothrops jararaca* venom.

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Introduction: Disintegrins are proteins isolated from venoms of several snake species. These proteins bind to different integrins present on cell membrane via a specific sequence of three amino acids. Integrins are involved in different biological process, such as cell-cell and cell-matrix binding, cellular differentiation and cell migration. Jararhagin-C is a toxin type-disintegrin containing the tripeptide ECD (Glu-Cys-Asp) on its binding site to integrins, isolated from *Bothrops jararaca* venom. Jararhagin-C possesses the feature of binding to α2β1 integrins present on the surface of platelets and also bind on integrins present on endothelial cells. **Objectives:** This study aimed to investigate the effects of Jararhagin-C on gene expression of human vascular endothelial cells (HUVEC). **Methods:** Initially it was verified thebinding capacity of jararhagin-C on HUVECsthrough inhibition of cell adhesion assay. Suspensions of HUVECs were incubated with jararhagin-C and seeded on well plates pre-coated with collagen I, IV and fibronectin. After washing steps, the adherent cells were quantified by MTT method. It was analyzed the time-course of 9 genes involved in wound healing process. The gene expression was evaluated by Real-time PCR on HUVECs previously adhered to collagen treated by jararhagin-C and compared with a control group of cells in the same condition. The fold change (Fc) was calculated by ΔΔct equation. **Results and Discussion:** The cell adhesion assay showed that jararhagin can slightly inhibit the cell adhesion to collagen I, suggesting that jararhagin-C bound itself on HUVEC. The time-course of gene expression showed an up-regulation of IL-6, CXCL-6, and MMP-10 (Fc = 3.0; 1.8 and 2.5 respectively) and down regulation of Il-8 (Fc=-0,8). At 6 hours the genes E-Selectin, I-CAM-1, IL-8, Angiopoetin-2 and MMP-10 were up-regulated (FC = 6.0; 7.0; 1.5; 2.0; 2.5 and 2.0 respectively) while IL-6 was down regulated (Fc = -0.5). At 24 hours we observed down regulation of IL-8, CXCL-6 and CD-69 (Fc= -0.5; -0.3 and -0.4 respectively). Our results suggest that jararhagin-C can bind to endothelial cells growing on collagen I, but does not bind to endothelial cells cultivated on fibronectin and collagen IV. Jararhagin-C induces up and down-regulation of genes expressed by endothelial cells involved with wound healing process. The jararhagin-C ability to bind to integrin α2β1, as well as collagen, is probably the responsible by the effects observed here.

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11.35 Evaluation of the interaction between bacteria and the toad *Rhinella icterica* skin by means of *in vitro* and *in vivo* methods

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Introduction: Currently, there are approximately 6,200 described species of anuran amphibians. These animals are still dependent on water for breeding and breathing, and their skin plays a key role in the protection against the adversities of the environment where they live. This moisturized skin also favors the growth of various microorganisms that are not always associated to the amphibian skin in a beneficial way. Actually, it is believed that many of these microorganisms are opportunistic and potentially pathogenic, and that they are important factors contributing to the amphibian population decline. **Objectives:** The evaluation of the interaction between bacteria and the toad *Rhinella icterica* skin performing *in vitro* and *in vivo* experimental infection and analyzing the results by means of morphological studies. **Methods:** The *in vitro* model consists in using a number of skin fragments obtained from one specimen of *R. icterica* and infecting each one of them with different bacterial species (*Citrobacter freundii* - strains 0014/3 and 0026/1; *Klebsiella pneumonia* - strains 0011/2 and 0038/1; *Escherichia coli* - strains 0011/1 and HB101, a non-pathogenic strain), for 24 and 48 hours, at 37°C. The bacterial strain presenting higher ability to adhere to the skin fragment is then selected for the *in vivo* infection assay, in which a group of three toads are experimentally infected for 7 days, and have samples of their skin collected, analyzed, and compared with skin fragments of a non-infected animal. All samples are morphologically analyzed by light (LM) and scanning electron microscopy (SEM). **Results and Discussion:** In the *in vitro* assay, all bacterial strains adhered very well to the surface of the toad skin, but adherence was more intense in fragments from the dorsal skin. The 48-hour infection, observed by LM, showed an uncommon spacing between epidermal and dermal skin layers, with the presence of groups of bacteria in some of these areas. SEM analysis showed skin regions where bacteria seemed to be penetrating the surface, suggesting a real ability for skin invasion. This possibility is now being investigated by transmission electron microscopy. Based on our results and on the fact that *C. freundii* has been largely associated with the amphibian skin disease known as red-leg syndrome, the strain 0014/3 has been chosen for the *in vivo* assay. Preliminary results showed that *C. freundii* was able to adhere to the skin but was not able to provoke skin lesion after a 7-day infection. Other bacteria concentrations and/or longer periods of infection will be tested for a more detailed evaluation of the infecting potential of *C. freundii* in the toad skin.

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11.36 Analysis of Antivenom Proteins on Bothrops jararaca Snake Plasma

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Introduction: The snake venoms are a complex mixture of proteins, nucleotides and inorganic ions, which confer toxic properties of these complex venoms. It is known that most venomous snakes have natural resistance against their own envenomation due to neutralizing potential of some plasma proteins. However, these resistance mechanisms are not fully elucidated. **Objective:** Thus, this study aims to characterize the plasma proteins from *Bothrops jararaca* responsible for protection against its own accidental poisoning. **Methods:** Venom and plasma was obtained from *B. jararaca* snake from Herpetology Laboratory of the Butantan Institute. The samples (plasma and venom) were maintained at -20 ° C until the time of analysis. Determination of protein concentration was performed by absorbance at 280 nm on a plate reader (Biotek-Epoch). The plasma was subjected to electrophoresis on 2-D SDS-PAGE. We performed the transfer of proteins to PVDF membrane for identification of spots of interest (Western Blotting). After transfer, the membrane was incubated with the venom of *B. jararaca*. Then this was incubated with antibothropic serum, followed by anti-horse serum labeled with peroxidase and developed with DAB and H2O2. **Results and Discussion:** Western Blotting revealed spots in the plasma of *B. jararaca* that bind to this, suggesting a protective role of these proteins against self- envenomation. Conclusion: The prospect of this work is an identification of plasma proteins that recognize venom proteins by mass spectrometry.

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11.37 Quantitative Proteomic Analysis of Adrenocortical Tumor Cells Stimulated with FGF2 through Spike-In SILAC

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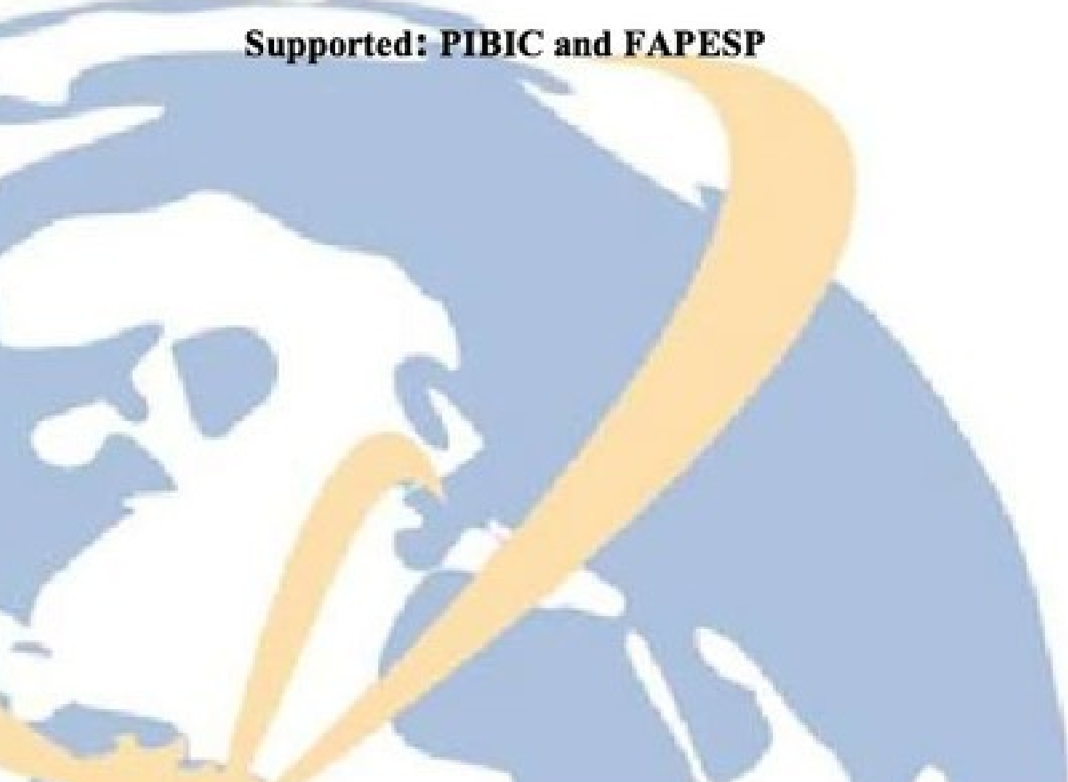
Introduction: In Y1 murine adrenocortical carcinoma cell line, the fibroblastic growth factor 2 (FGF2), that induces proliferation and carcinogenesis, promotes G0/G1 transition but delays S-phase and permanently block cells in G2/M. To better understand the molecular mechanism induced by FGF2 in cell cycle we perform quantitative proteomics analysis through a method called Spike-In SILAC. This approach is based on the incorporation in cell culture of a stable aminoacid isotope heavier than the natural isotope (SILAC method) and the labeled peptides can be used as an internal standard in different experimental conditions. By mass spectrometry analysis, it is possible to differentiate the ions from labeled and non-labeled cells and evaluate its relative amount based on the extracted ion chromatogram of each ion. Using a single computational pipeline developed by Max Planck Institute of Biochemistry, we can identify and analyze proteomic data from different experimental conditions at once, saving time and improving results reliability. **Objectives:** To analyze the metabolical response in Y1 cells stimulated with FBS (fetal bovine serum) and FGF2 through quantitative proteomics (Spike-In SILAC) in three different time points with three biological replicates. **Methods:** Y1 cells grown in DMEM medium were harvest for 48 h and stimulated with 10% of FBS or 10% FBS+10 ng/ml FGF2 for 0, 3 and 5h. Proteins fromY1 cells previously grown in SILAC medium (DMEM medium with $^{13}C_6$-Lys supplemented with dialyzed fetal bovine serum) were obtained and used as internal standard (Spike –In SILAC). Labeled proteins (SILAC) were mixed in 1:1 with non-labeled cells, digested with trypsin and analyzed by a high-resolution mass spectrometry LTQ-Velos Orbitrap (Thermo) in a 2.5-hour acetonitrile gradient length. The data were processed by Max Quant program using Uniprot MOUSE database, 6 ppm MS tolerance, 0.5 Da MS/MS tolerance and 1% FDR rate. The data were compared and statistically analyzed by Perseus. **Results and Discussion:** We identify about 1100 proteins groups, which approximately 40% were quantified. From these, after filtering procedures we obtained around 40 proteins differentially expressed (ANOVA test with 0.1 of false discovery rate) whose 17 and 14 are related to acetylation and binding process, respectively. We are currently working both on detailing the functionality of those recent discoveries and also on increasing the number of identified and quantified proteins.

Supported: PIBIC and FAPESP



11.38 Behavior of atypical enteropathogenic *Escherichia coli* (aEPEC) from diferente serotypes in the interaction with macrophages

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Introduction: We identified two isolates of aEPEC (LB7 and BA320), both from serotype O55:H7, which are very poorly phagocyted by macrophages.Experiments performed with fractions obtained from solid phase extraction and reversed phase HPLC fractionation of the culture medium from these samples reduced phagocytosis of control bacteria, yeast and latex particles. So far, we do not know whether the antiphagocytic capacity observed in these samples is related exclusively to serotype O55: H7. **Objective:** The aim of this study was to investigate the behavior of aEPEC strains from the classic serotypes in the interaction with macrophages. **Methods:** J774.A1 macrophages were infected with aEPEC LB5 (O26:H11), LB12 (O119:H2), LB13 (O11ab:H9), LB14 (O55:H7), BA487 (O55:H7), BA580 (O119:H2) and BA4147 (O55:H7) for 10, 30 and 60 min. aEPEC LB7 was used as o a positive control and typical EPEC 28 (O55:H6), highly phagocytosed, was used as a negative control. The aspects observed in this interaction were the percentage of infected macrophages and the number of colony forming units (CFUs). **Results and Discussion:** The percentage of infected macrophages byaEPEC samplesLB5, LB13, LB14 and BA580 was similar to LB7, i.e. 33%, 27% and 20% after 10, 30 and 60 min respectively. The number of LB5, LB13 and LB14 CFUs was also similar to the positive control. The remaining samples (LB12, BA487, BA4147), including sample BA580, which showed a low percentage of infected macrophages, produced a high number of CFUs, after only 10 min of infection, similar to the negative control. The analysis of both the number of infected cells and the number of intracellular bacteria is extremely important in the study of bacterial behavior in the interaction with professional phagocytes. The results obtained with sample BA580 illustrate this, considering that although it infects a small percentage of cells, the amount of intracellular bacteria is high, suggesting that it doesn't prevent phagocytosis. In addition, the determination of the percentage of infected macrophages, although high, may not reflect increased phagocytosis, since through light microscopy it is not possible to discern internalized bacteria from adhered ones. The results presented here suggest that other aEPEC serotypes, other than serotype O55:H7, might also present an anti-phagocytic capacity, as two of the three less phagocyted strains in this study are from serotypes O26:H1 and O111ab:H9, which are also considered as classical. These results suggest that aEPEC from different serotypes may induce the anti-phagocytic effect. The presence of anti-phagocytic compounds in the culture supernatant of these samples will be investigated in future works.

Supported by INCT-Flx-Cx, CNPq/PIBIC

11.39 Occurrence of *Arisocerus hertigi* (Brennan & Jones, 1964) (Trombiculidae) in an atlantic forest area

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Introduction: The last revision of the family Trombiculidae in the Neotropical and Neartic regions was published during in the 80's, 87 genera were recognized, with the inclusion of *Arisocerus,* described in 1970. The genus includes two species recordedin the Neotropics: *A. amapensis* Brennan, 1970 and *A. hertigi* (Brennan & Jones, 1964). The geographical distribuition of the first includes Brazil – State of Amapá (Serra do Navio, infesting *Oryzomys macconnelli*, *Oryzomys capito* and *Proechimys guyannensis*) and Pará (Belém and Bragança, infesting *Oryzomys capito*); Suriname (Baboenhol, Brownsberg and Santo Boma, infesting *Proechimys guyannensis*; River Tapanahoni, infesting *Oryzomys laticeps* and *Myoprocta acouchy*); Venezuela (Bolivar, infesting *Proechimys guyannensis).* The second species was described from a "cerrado" area in Brazil, Distrito Federal – Brasilia infesting the marsupial *Didelphis albiventris* and Paraguay (Sommerfiel, infesting agouti and opossum). **Objectives:** New records for locality and host of *A. hertigi* from Brazil. **Methods:** In this study, specimens of chigger mites were obtained infesting wild rodents collected in the Parque do Estado, São Paulo in 2010. Some were mounted on slides for morphological studies and the remaining ones were prepared for molecular analysis. **Results and Discussion:** Morphological studies demonstrated that the diagnostic characters for *A. hertigi* are: *Idiosoma:* with one pair of humeral setae; dorsal setae 2-6-6-2-2; SIF: 7BS-3N-3111.1000; anus in the third row of ventral setae, sternals setae 2-2 plus two ventral setae, one pair of para-anal setae and one pair of postanal setae similar to dorsals. *Gnathosoma*: conspicuously punctuated; cheliceral blade with a trifurcated apex; palpal setae B/B/NNN; papal claw 3-pronged. Scutum: moderately punctuated with sinuous margins; sensila strongly expanded and asymmetric with large setae bordering only the anterior margin; posterior lateral setae long; PL>AL>AM; eyes 2/2 in an ocular plate. *Legs:* 7-7-7; coxae unisetose.Molecular studies are still in being carried out, however, based on the morphological studies, this is the second record of the species for the country and the first for the Atlantic Forest and for Cricetidae rodents.

Supported by CNPq, FAPESP

11.40 Distribution of araneomorph soil spider fauna at different altitudes in Serra do Japi, state of São Paulo, Brazil

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Introduction: Seven percent of all species of living organisms described as new in this century belong to the Class Arachnida, and, of these, 44.9% are spiders. The spider taxonomy as well as various aspects of the biology and ecology of spiders are little known. The geographic distribution of species, patterns and environmental variables responsible for these patterns only began to be investigated. The understanding of these patterns is old goals of ecology and biogeography. The project investigates the altitudinal distribution of araneomorph soil spider species in the Serra do Japi, state of São Paulo, in a Brazilian Atlantic forest area, regarding the species composition and richness. For this, the araneomorph soil spiders collected by means of pitfall traps in four seasons during the year of 2009, in two distinct points of three different altitudes (800 m/1000 m/1200 m) are being identified, quantified and species richness indices for each point will be obtained through different estimators. **Objectives:** To inventory the fauna of araneomorph soil spiders in the Serra do Japi, to describe any new species found, to evaluate the influence of altitude on richness and species composition of araneomorph soil spiders in the area. **Methods:** Samples were obtained using pitfall traps made of 500 ml disposable cups with 100 ml of etanol and 10% formaldehyde. In each point the traps were arranged in five rows of ten, and remained in each area for 8 full days. They were distributed in six points, in three different altitudes: area I: 846 m, area II: 826 m, area III: 1200 m, area IV: 1177 m, area V: 1054 m, area VI: 1037 m. **Results and Discussion:** Twenty six families of araneomorph soil spiders were obtained. This number is very close to that found for other Atlantic forest areas of São Paulo, i.e. 26 families in a region in the south of the city of São Paulo, and 25 families in four areas on the west side of the same town. The identification of families was completed and the identification of genera and species is being done. Only after this stage it will be possible to have the information on the composition and species richness of the Serra do Japi and to calculate the species richness indices. Comparisons between the species composition and richness among the different altitudes of the Sierra also will be possible only after the completion of the identifications. Until now, individuals of some families have been found only at some altitudes, as for example Amphinectidae (1200 m), Anyphaenidae (1000 m), Caponiidae (1200 m), Nesticidae (1200 m) and Synotaxidae (800 m). However, the number of individuals of these families is too low in order to draw any conclusion.

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11.41 Knowing the Visitors at the Biological and History Museums of Instituto Butantan

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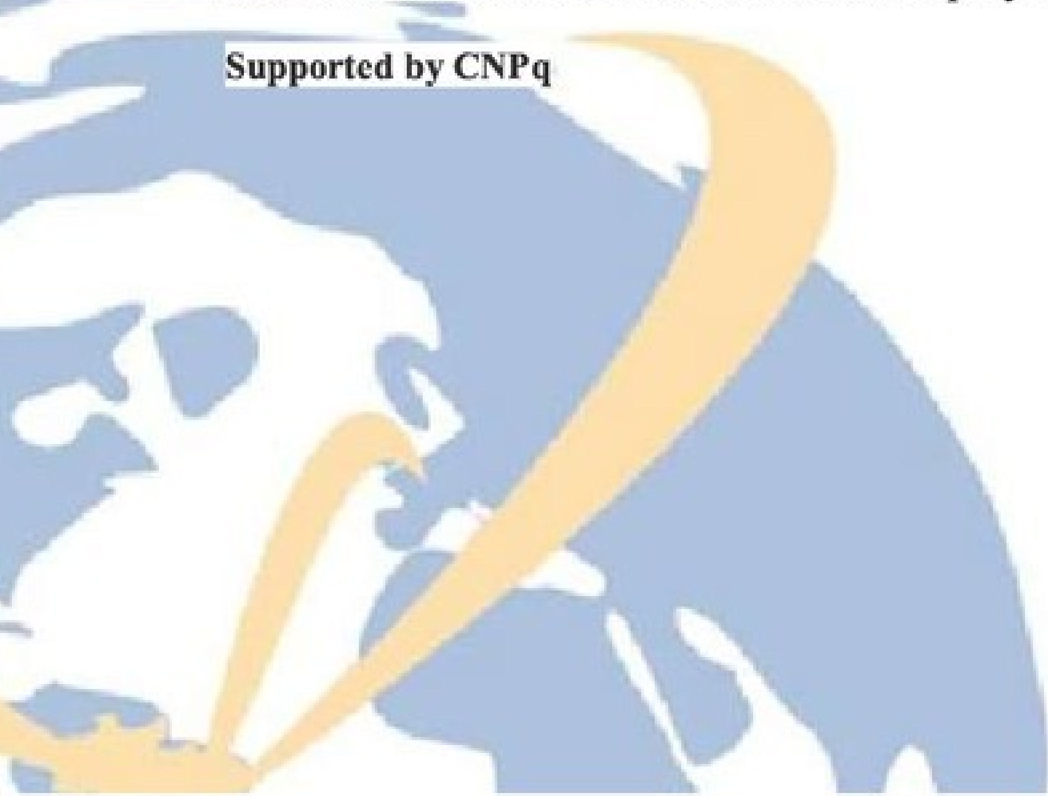
Introduction: Visitors' studies in museums are a fundamental tool to the evaluation of exhibitions and the comprehension of the museum experience. Understanding the visitors' perceptions about a specific exhibition may provide invaluable information about the understanding of the contents, attractiveness of the exhibition, and patterns that motivate further visits to the museum. Cultural profile, social context of the visit, and time spent in the museum, among other factors, can influence visitors' behavior. The visitor expectations prior to the visit may be fulfilled or not, and in this case it might result in frustration thus disturbing the understanding of the exhibition. The two museums investigated are located in the Butantan *campus*, a green area with several services. **Objectives**: The main objectives are to describe and analyze the profile of two Butantan Museums' visitors and to identify the elements of major attractiveness in the exhibits of the Biological and History Museums. **Methods**: A preview survey was conducted at the Butantan Park to identify the visitors' profile, whether they have visited or not the Museums. Visitor's behavior was recorded at these two Butantan museums, while post visit interviews were conducted with an aleatory sample. The questions were elaborated with the objective of finding out what are the visitors preferences in relation of exhibition and how they understand the main concepts presented. There were also questions about visiting habits to others museums and science centers, beyond sociodemographic profile. Timing and tracking was made, observing the stops, the route of the visitor in each museum exhibit. A total of 1150 interviews in the Park and 232 interviews and 200 timing and tracking registration in two museums (Biology and History) constitute the dataset analysed. **Results and Discussion:** Results of the visitors' interviews at the Biological Museum showed that most of the public consists of white women between 35 and 44 years old, with high level education. Visitors usually come with companions and children, reside in the city of São Paulo and often visit museums. Results are similar to those obtained in other similar researches, as the one promoted by OMCC in 2006 – 2007 in 13 museums and the one held at the art Museum Lasar Segall in 2001 (both in the city of São Paulo). The data collected in the Instituto Butantan confirms that the museum is a place of social interaction especially of families aiming to have leisure and to learn. The maps of the visitors' routes and behaviors combined with the interviews provide data about the attractiveness of the exhibits and the displays that held most of the attention.

Supported by CNPq



11.42 Allergy to *Loxosceles* spider venom as occupational disease in arachnologists

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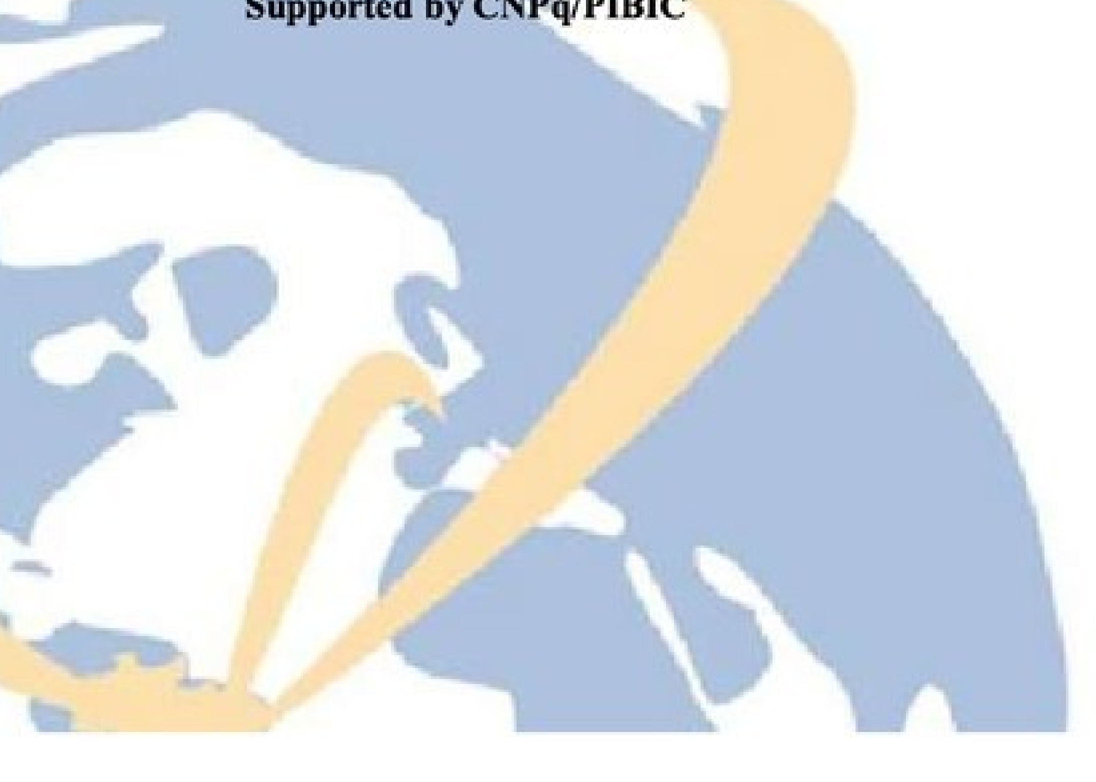
Introduction: Reports on allergic reactions from spiders are rare. To our knowledge, there are no reports associating spiders of the genus *Loxosceles* with allergic reactions. Moreover, workers of the Butantan Institute have complained of allergic symptoms when in contact with their venoms. **Objectives:** The aim of this work is to investigate the prevalence and predictors of venom allergy among these workers and to confirm the involvement of IgE-mediated mechanisms. **Methods:** All subjects completed a physician-administered questionnaire containing questions regarding their personal history of allergy, spider bites, and contact (oral or ocular) with spider venom, as well as their work history (length of employment and specific work tasks) and work-related symptoms. The job of arachnologists entails specific tasks, including maintenance of spiders in captivity, spider venom extraction, and the handling of spider venom. The presence of venom sensitization will be determined through quantification of specific IgE and IgG (ELISA). Allergens will be studied using the Western blots assays. **Results and Discussion:** Of the 16 subjects evaluated, 11 (68.8%) were female. The mean age was 41.0 ± 14.8 years, and the median age was 39.0 years (interquartile range 28.2–55.2). The median length of employment was 7.2 years (interquartile range 1.3–22.5). Of the 16 subjects interviewed, 7 (43.8%) were considered atopic. Moreover, twelve subjects (75.5%) presented allergic symptoms ranging from urticaria, rhinoconjuctivitis and asthma to anaphylaxis, when exposed to spiders of genus *Loxosceles* (or its venom). High levels of total IgE (> 100 UI/L) was observed in 7 workers (43.8%). Specific IgG antibodies could be observed in 11 subjects (68.8%). None individuals had specific IgE antibodies to *Loxosceles* venom. In three workers there was recognition of some components of the venom (Western blotting). Allergic symptoms were associated with specific tasks, primarily the maintenance of spiders in captivity (P = 0.018), but not with exposure level (P = 0.558), specific IgG (P = 0.725), high levels of total IgE (P = 0.217), and atopy (P = 0.608).The prevalence of symptoms related to exposure to *Loxosceles* spiders or its venom was 75.0% in workers of the Laboratory of Arthropods. The only predictor that showed statistically positive relationship with the presence of these symptoms, so far, was the task of maintaining the spiders in captivity (p = 0.018). Specific IgG to the venom of *L. gaucho* was observed in 68.8% of workers, indicating probable sensitization by inhalation. It has not been possible to prove the IgE-mediated mechanism in the genesis of allergy symptoms reported by workers.

Supported by CNPq/PIBIC



12 PAP Program

12.01 Antiviral activity from eggs waxes from the tick *Rhipicephalus (Boophilus) microplus*

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Introduction. Ticks lay their eggs in the environment, and involve the eggs in a waxy layer to protect them from desiccation and microbial attack. This wax is produced by an organ known as Gené's Organ. Bio prospection has shown the presence of active principles in the hemolymph of arthropods as well as in the salivary glands of ticks. Some of these compounds are of great interest for the development of new pharmacological drugs. In this study, different tick species from colonies maintained in our laboratory, were used to test the antimicrobial effect of the polar extract present in the composition of the wax. **Objectives:** The objective of this study was to evaluate the antiviral effect present in the extract of the wax that involves the eggs of some tick species, in this study the wax from *Rhipicephalus (Boophilus) microplus*, as well as to evaluate the *in vitro* toxicity of this extract. **Methods:** The egg masses were extracted with icy cold 6.8 phosphate buffer and tested against Influenza (H_1N_1) to determine the antiviral activity of the tick eggs wax. MDCK cells were infected with influenza viruses. Culture of MDCK cells, performed in 96 wells microplate, was treated with 2600, 1300, 650, 325, 162.5, 82, 41 and 20.5 μg/mL of the eggs wax extract 1 h before infection. After 72 h post infection cytopathic effect induced by the virus was observed, the culture medium was removed and the cells in the plate were stained with crystal violet (0.2% in 20% methanol). The egg wax was maintained in culture during the time of infection. **Results and Discussion:** Amounts as small as 325 μg/mL of the extract were able to inhibit the replication of the virus. Besides, the sample presented very low citotoxicity on Vero cells. This result is accordance with prior results from the wax of *Amblyomma cajennense.*

Supported by: FAPESP, Fundação do Desenvolvimento Administrativo (FUNDAP)

12.02 Recombinant Pro-Domain of Snake Venom Metalloproteinases Inhibits Venom Toxic Activities

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Introduction: Snake Venom Metalloproteinases (SVMPs) are zinc-dependent enzymes that hydrolyze extracellular matrix and plasma proteins. In *Bothrops* envenomings, SVMPs contribute to local and systemic effects, especially induction of hemorrhage. SVMPs are synthesized as zymogens and their latency is controlled by the presence of a pro-domain, via a cysteine-switch mechanism, similarly to the mechanisms involved in the activation of Matrix-degrading Metalloproteinases (MMPs). SVMP zymogens are not found in venoms, but a number of pro-domain peptides have been characterized in *Bothrops jararaca* venom peptidome.**Objectives:**Test the inhibitory activities of the recombinant pro-domain of jararhagin (PD-Jar) and a pro-domain synthetic peptideagainst toxic activities of*B. jararaca*venom and isolated SVMPs. **Methods:** PD-Jar was produced in *E. coli*under standard protocols,solubilized in 6M urea and refolded in column (Immobilized Metal Affinity Chromatography). Pro-domain peptideENVEKEDEAPKMCG-NH2 (detected in the peptidome and present in pro-domain structure) wasprepared by Fmocsolid-phase peptide synthesis. Samples of *B. jararaca* venom, BnP1 and jararhagin were pre-incubated with different proportions of PD-Jar or synthetic peptide and tested for residual fibrinolytic activity, in agarose/fibrinogen plates, or hemorrhagic activity, after intradermal injection on the dorse of mice.**Results and Discussion:**PD-Jar was obtained as a major 21 kDaSDS-PAGE band, yielding17 mg/L culture.In tests of fibrinolytic activity, PD-Jar inhibited 78%whole venomactivity,and totally inhibited BnP1 and jararhagin atinhibitor to enzyme ratios of4:1 (w/w) and 6:1 (w/w), respectively. Regarding hemorrhagic activity, PD-Jar was able to completely inhibit the action of*B. jararaca*venom and jararhaginat inhibitor to enzymeratios of 5:1 (w/w) and 10:1 (w/w), respectively. Pro-domain-derived synthetic peptide also inhibited fibrinolytic and hemorrhagic activities of jararhaginat aconcentration of*ca.* 1 mM. The results show the efficacy of recombinant PD-Jar as an inhibitor of SVMPs' most important activities and identify an inhibitory peptideas the minimal structure responsible for pro-domain activity. Considering the conserved properties of catalytic motifs in SVMPs and MMPs, our data indicate the potential role for PD-Jar or pro-domain derived peptides as metalloproteinase inhibitors withpotential therapeutic applications.

Supported by: Fundação do Desenvolvimento Administrativo (FUNDAP), FAPESP, CNPq, PAPES VI/FIOCRUZ and INCTTox/CNPq.

12.03 Blood Transfusion in Snakes

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Introduction: In recent years transfusion medicine, as well as immunohematology studies, have become essential for the treatment of various diseases, which encouraged the development of new technologies and better understanding of the use of blood and its components. Currently, blood transfusions are increasingly common in veterinary medicine and is often used in emergency and surgical procedures. The main indications are life-threatening severe anemia, immune-mediated diseases and severe non-regenerative neonatal isoerythrolysis. In snakes the principle indication for blood transfusion is severe anemia due to internal bleeding caused by traumas and caquexia. The determination of haematological and biochemical parameters is essential to estimate the health status of the snakes recently-caught in nature and those maintained in captivity. Blood typing and compatibility tests are used to prevent incompatible blood transfusion that can cause severe immune-mediated transfusion reactions that endanger the patient's life. **Objectives:** Study the vertebral venous plexus as an alternative via to collect large blood volumes; implement catheterization techniques in snakes and study blood transfusion in snakes. **Methods:** Five healthy boas (males and females) from the Laboratório de Herpetologia, with average weight of 3577.5 g were used as donors. Blood was collected slowly by the boa's vertebral venous plexus with the aid of 22G scalp and put into suitable pediatric blood bags. The receptors were ten healthy recently-caught rattlesnakes (5 males and 5 females) with average weight of 707g. The rattlesnakes were divided into two groups: experimental group (3 males and 3 females) and control group (2 males and 2 females.) The day before the catheter implantation surgery, blood tests were performed to determine the hematocrit value and health state of the animals. After the catheterization, the volume of blood necessary to lower the hematocrit levelto 10% was collected and the same quantity of blood from the donor was tranfused (CEUAIB: 1009/13). **Results and Discussion:** The vertebral venous plexus was successfully used for the collection of large blood volumes, both in boas and rattlesnakes. The catheterization technique was successfully performed, being an excellent mean for blood transfusion. The blood transfusion showed considerably significant results in the increase of hematocrit values in the animals that received blood transfusion after the first 24 to 48 hours. In the first 24 hours the result was even more significant than in the first 48 hours after transfusion. Comparing the hematocrit of the first 24 to 48 hours on both groups, there was a significant decrease in the hematocrit values of the control group.

Supported by Fundação do Desenvolvimento Administrativo (FUNDAP)

12.04 Development in Captivity-Born*Bothrops jararaca* Subjected to Two Different Feeding Techniques.

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Introduction: Protein deficiency is a frequent problem in snakes that do not feed due to captivity stress or pre-existing diseases and, although being a stressor, force-feeding is sometimes necessary to maintain the animal alive. To perform force-feedingin poisonous snakes, the animals are placed in a container saturated with carbon dioxide gas (CO_2). The use of CO_2 is allowed by the Ethic Committee on Animal Use atInstituto Butantan and besides diminishing the regurgitation rate, also increases the security of the professional performing the procedure. **Objective:** Compare two different feeding techniques in relation to weight gain and length increase. **Methods:**Thirty two captivity-born*Bothrops jararaca* (9 males and 23 females) were used in this study.The snakes were divided into two groups: control group (6 males and 14 females) and experimental group (3 males and 9 females). The animals of the control group were fed every 15 days during the first year and every 30 daysin the second year with 10% of their body weight in live mice, while the animals of the experimental group wereforce-fed with exactly the same weight ratio. To perform the force-feeding technique, snakes were "sedated" in a container saturated with carbon dioxide. Mice were submitted to euthanasia,their incisors cut and they were immersed in vitamin solution to facilitate their way through the snake's digestive tract. Both groups were measured routinely.**Results and Discussion:**In the first month of study there were no statistical differences between the groups but in all the following months extremely significant differences were seen in weight gain ($p<0,000$) and length increase ($p<0,000$). After 30 months, the control group increased its weight in 370% and its length in 111%, while the experimental group in 282% and 89%, respectively. With these results, we can suggest that the force-feeding technique, although sometimes necessary, is stressful and impairs the normal development of the snakes. At the end of the study, the average weight of the control and experimental groups were 50g and 39g, respectively and the average snout-vent length of the control and experimental groups were 59cm and 52cm, respectively.

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12.05 Adverse reactions following antivenom use in patients treated at Vital Brazil Hospital in the period 2002-2012

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Introduction: Antivenom, the serum of animals immunized with venom, is the only specific treatment for poisoning with snakes and spider venoms. However, some patients quickly develop cutaneous or systemic anaphylaxis when it is given. Pre-medication has been used to protect against early adverse reactions (EAR) following antivenom administration. Studies have evaluated its efficacy with variable results. **Objectives:** Analyze the presence of EAR following antivenom administration and the relationship with use of pre-medication and dilution of antivenom. **Methods:** We analyzed 1279 patient records treated at HVB in the period from 2002 to 2012 who received antivenom due to accidents by snakes of the *Bothrops* and *Crotalus*, and spiders of the genus *Loxosceles*. The main variables analyzed were: pre-medication (corticosteroids, antihistamines H1 and H2), infused amount of antivenom, antivenom dilution, and presence of EAR. **Results and Discussion:** Of the 1279 cases analyzed, 1071 (83.7%) were caused by snakes of the genus *Bothrops*, 38 (3.0%) by snakes of the genus *Crotalus*, and 170 (13.3%) by spiders of the genus *Loxosceles*. Of the 168 (13.1%) patients who developed reactions, 125 suffered accidents caused by snakes of the genus *Bothrops* (11.7%), 11 by snakes of the genus *Crotalus* (28.9%) and 32 by spiders of the genus *Loxosceles* (18, 8%). The main symptoms were: skin lesions (57.7%), cough (29.8%), nausea and vomiting (22.6%), and pruritus (21.4%). The use of antivenom prior ($p = 0.562$), number of vials used ($p = 0.365$) or the dilution of antivenin ($p = 0.609$) were not associated with the presence of EAR. There was no relationship between the absence of EAR with premedication with corticosteroids ($p = 0.378$) or anti-histamine H2 ($p = 0.394$). However, there was with the use of antihistamine H1 ($p = 0.049$).EAR were more frequent with use of serum anticrotalic. The number of vials and dilution did not influence the presence of these reactions. The use of pre-medication does not seem to have an effect on the prevention of EAR, except perhaps the use of antihistamine H1.

Supported by Fundação do Desenvolvimento Administrativo (FUNDAP)

12.06 Cloning, expression of bivalent single chain variable (scDbs) and disulfide stabilized (scdsFvs) Fv fragments anti-crotoxin

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Introduction: Variable Fragments (Fvs) are heterodimers with only the V_H and V_L domains of antibodies, which contain structural information necessary for specific antigen binding. Because of their small size, they can be easily submitted to genetic manipulation and also used in applications requiring faster penetration, such as tissues and tumors. However, in most cases, only V_H-V_L domains are too weak to maintain a stable and functional Fvs, which results in a tendency to rapid dissociation. In order to solve this problem V_H and V_L has been joined by a flexible peptide linker, such as $(G_4S)_3$.A reduction of this linker to $(G_4S)_2$ promotes generation of bivalent scFvs, known as single-chain diabodies (scDbs). Another possibility to eliminate the problems of aggregation is to add a disulfide bridge between V_H and V_L, generating the disulfide-stabilized single-chain Fvs (scdsFvs). The production of Fvs against a toxin (crotoxin) from *Crotalus durissus terrificus*, may represent an alternative ophidian therapy. **Objectives**: To generate scDbs and scdsFvs from a scFv against crotoxin in order to enhance its stability, efficiency and expression in *E. coli*. **Methods:** Two synthetic genes were designed and produced by Geneart based on the sequence of a scFv against crotoxin: scDbs with V_H and V_L linked by $(G_4S)_2$ and scdsFvS with glycine and threonine replaced by cysteines on V_{H44} and V_{L100}, respectively. Both constructs were cut with BamHI and HindIII restriction enzymes and inserted into pET20b+ vector. Next, the clones were selected by PCR and submitted to sequencing. The construction containing scDbs or scdsFvs were used to transform *E. coli*bacteria C43(DE3).Antibodies were expressedusing1mM of IPTG for induction and growth at 37° C for 4 hours. The samples were purified by immobilized metal affinity chromatography (GE healthcare) following the manufacture´s orientation. **Results and Discussion:**The clone sequencing confirmed the presence of cysteine residues at positionsVH44 and VL100 on scdsFvs and the correct shorter linker on scDbs. Currently, the expression of recombinant antibodies is under optimization in bacteria. Those modified scFvs may represent promising candidates to be tested regarding their neutralizing ability against the toxic effects of crotoxin.

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12.07 7th Introductory course to the "Programa de Aprimoramento Profissional (PAP) da Secretaria de Estado da Saúde no Instituto Butantan"

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Introduction: The "*Programa de Aprimoramento Profissional* (PAP)" created in 1979, aims to complement the formation in the health area. In Butantan Institute (IBu), this program lasts 1 or 2 years and is composed of 40 hours/week of activities. Since 2007, before the beginning of their laboratory activities, the students have to attend a course organized by a committee composed by researchers from different laboratories. In this course, pertinent themes are ministered by scientific researchers, specialists and also students of the second year of the program. The course focuses on the integration of the grant-holders to the different areas of the Institute. At the end of the course, students have to answer an anonymous questionnaire which gives them the opportunity to evaluate it and to express their point of view about its activities. **Objectives**: The aim of this work is to describe and evaluate the planning, organization, and application of the sixth PAP-course, offered in 2013. **Methods**: The activities lasted 60 hours and were divided into participative and theoretical classes, distributed into an 8 hours-day journey. All Divisions of the IBu, Museums and Collections were introduced to the students, and theoretical classes concerning several important topics were presented, such as: equipment operation, laboratory safety, animal care and ethics. At the end, a questionnaire was answered by the students, to know their opinion about the course. **Results and Discussion**: In 2013, 45 just-graduated with different formations were received by the program (1 Biotechnology; 1 Historian, 27 Biologists, 4 Pharmacists; 4 Biomedical, 4 Veterinary) and 77% did not belong to IBu. The questionnaire was answered and the program was considered totally satisfactory to all of them, as well as the content and workload. The helpfulness and attention of teachers and coordination staff were totally satisfactory to them. The main subjects of interest were Bioethics, Biotherium and Scientific Writing; on the other hand the History and the Organogram of the Institute were pointed as areas of medium interest. The feedback received can play an important role in organization and improvement of the course in future years. The high level of student's satisfaction suggests that they were academically benefited by the course and indicates that they were stimulated to develop their own scientific projects during their specialization traineeship.

All the authors equally contributed to this work.

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12.08 Influence of preconditioned media (MPC) on interaction of human and animal enteropathogenic *Escherichia coli* (EPEC) strains with HEp-2 cells

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Introduction: Enteropathogenic *Escherichia coli* (EPEC), a major causative agent of diarrhea in children in developing countries, can be classified as typical or atypical EPEC and can be found in different animal hosts. The hallmark of its pathogenesis is the formation of the "attaching and effacing" (A/E) lesion in the intestinal epithelium. The proteins involved in A/E lesion are encoded by genes located in a pathogenicity island, named "locus of enterocyte effacement" (LEE). Some studies suggest that LEE genes are regulated by quorum sensing, and probably the signals involved in this regulation are molecules produced by EPEC, as well as the host, the resident microbiota and transient species. To date, four quorum sensing systems were described, the ones that use autoinducer-one (AI-1) and autoinducer-3 (AI-3) are found in Gram-negative bacteria, Gram-positive uses a polypeptide autoinduction (AIP) and the system that uses autoinducer-2 (AI-2) is found in both, Gram-positive and Gram-negative bacteria, and may represent an interspecific signaling system. **Objectives:** The aim of this study was to verify the influence of animal EPEC to human EPEC strains, and vice versa, on adherence. **Methods:** We analyzed the adherence pattern to HEp-2 cells from E2348/69 (human EPEC prototype strain) and Ap155 (animal EPEC strain) wildtype, deleted in *luxS* and complemented with *luxS* in DMEM and MPC from both wildtype and mutant strains. **Results and Discussion:** In the control adherence assay with DMEM, the mutant strains showed a loss on the efficiency to form the characteristic microcolonies found in typical and atypical EPEC. Surprisingly, after 2h of bacteria - epithelial cells interaction, it was possible to verify that regardless the MPC tested, both wildtype strains showed a reduced adherence on HEp-2 cells. On the other hand, the *luxS* deleted strains showed an adherence pattern similar to the wildtype in DMEM. These results demonstrate that there is a possible communication between animal and human EPEC strains, and MPC seems to harm the growth of wildtype strains but is also capable of restore the *luxS* phenotype.

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12.09 A preliminary inventory of snake fauna in Belterra (Pará, Brazil)

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Introduction: Inventories of snake fauna, even the preliminary ones, show the importance to know which and how many species exist in a certain region. In the west of the State of Pará (Brazil), there are few studies regarding thesnake inventory, which shows the need for studies that compile the list of snake species in the region. **Objectives**: The objective of our study was to compile a list of snake species from the municipality of Belterra, in the west of the State of Pará (Brazil), in the urban area and around the Área de Proteção Ambiental de Aramanaí. **Methods**: To compile the list of species, we used material deposited in the herpetological collection of Faculdades Integradas do Tapajós (Linha de Pesquisa em Herpetologiana Amazônia LPHA-FIT), and material donated to Unidade Mista de Saúde de Belterra from 2005 to 2013. We analyzed 101 specimens and recorded 37 species of snakes of 27 Genera and 6 Families (Aniliidae, Boidae, Colubridae, Dipsadidae, Elapidae and Viperidae). **Results and Discussion**: The most abundant species from LPHA – FIT were: *Helicops polylepis* (n=27), *Bothrops atrox* (n=9) and *Oxybelis fulgidus* (n=7). Dipsadidae was the richest family (79,17%), followed by Viperidae (13,54%), Colubridae (5,02%), Aniliidae (3,13%), Boidae (2,08%) and Elapidae (2,08%). Nineteen species were represented by one single individual collected: *Boa constrictor*, *Corallus hortulanus*, *Eunectes murinus*(Family Boidae); *Chironius scurrulus*, *Drymarchon corais*, *Pseustes sulphureus*, *Tantilla melanocephala* (Family Colubridae): *Drepanoides anomalus*, *Erytrolampus aesculapii*, *Leptodeira annulata*, *Oxyrhopus melanogenys*, *Oxyrhopus trigeminus*, *Philodryas viridissima*, *Pseudoboa coronata*, *Pseudoboa neuwiedii*, *Siphlophis cervinus*, *Siphlophis compressus*, *Siphlophis worontzowi* and *Xenodon severus* (Family Dipsadidae): *Micrurus hemprichii*, *Micrurus spixii* (Family Elapidae) and: *Bothrops taeniatus* (Family Viperidae). According to our data, four out of 37 species in the region are venomous, belonging to the genus *Crotalus, Bothrops* and *Micrurus*. The species *Helicops tapajonicus* (n=3), with specific epithet referring to the type-locality (Tapajós River) and known only in Belterra (Pará, Brazil), has few natural history data. Information on species distribution was based on literature. Further field studies are necessary in order to broaden the knowledge and review the taxonomy of the snake fauna in the region.

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12.10 Blood transfusion in horses: adverse reactions

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Introduction: In critical situations of hypovolemia, system imbalances clotting or bleeding, the life of the horse needs a rapid transfusion fluids, whole blood or its fractions, such as platelets or plasma. The most important indications for the transfusion of blood horses are neonatal foals, trauma with large amounts of blood, hemolytic anemia and internal bleeding. **Objectives**: Review the adverse reactions during blood transfusions in horses and seek ways of prevention and treatment. **Methods**: a general review of the cross-matching in equine blood transfusion was based on national and international literature in last five years. The ideal donor would be a horse under three years of age who have not previously received a blood transfusion. There are seven equine blood groups defined: A, C, D, K, P, Q and U, and more than 400,000 antigenic variants, Aa and Qa antigens are reported to be the most antigenic in horses. Despite numerous indications for the administration of equine plasma in patients in intensive care, there is a potential risk of sudden severe allergic reactions and anaphylaxis, presenting with severe hypotension, shock or even sudden death. The risk of immune-mediated reactions during transfusion is minimized ensuring compatibility between blood donor and blood recipient. The cross-matching test is a relatively simple procedure for checking the compatibility of blood groups. To this end, it is possible to perform two tests: the main test verifies the compatibility of the donor cells with recipient's serum whether the secondary receiver has antibodies against the donor cell. The non-immune-mediated transfusion reactions rarely occur in horses, and usually result from problems with the collection, handling or administration of whole blood. It is likely that present in the cells contributing to the plasma transfusion reactions. The plasma prepared by gravitational sedimentation contains a larger number of erythrocytes and leukocytes compared to that obtained by plasmapheresis and centrifuged preparations. Leukocytes can fragment during storage and desgranular, pyrogens and other substances releasing pro inflammatory cytokines in addition to the donated blood or anti-leukocyte antibodies present in the plasma. Haemolytic transfusion reactions should be managed by cessation of the transfusion and maintenance of intravascular volume and perfusion with crystalloids and/or colloids. **Results and Discussion**: It is recommended, therefore, the cross-matching test before the blood transfusions in horses to prevent potential transfusion reactions. More research is needed in this area to develop safer and accessible methods.

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12.11 Production of EspA recombinant protein

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) are among the most important pathogens causing persistent diarrhea in children and adults worldwide. EPEC is among a group of pathogens that is capable of forming Attaching and Effacing (A/E) lesion as consequence of infection. This group includes enterohemorragic *Escherichia coli* (EHEC), rabbit diarrhoeagenic *Escherichia coli* (RDEC), the murine pathogen *Citrobacter rodentium,* and *Hafnia alvei*. Enteropathogenic *Escherichia coli* (EPEC) have been classified into two subgroups: typical EPEC (tEPEC) and atypical EPEC (aEPEC), based on the presence or absence of the EPEC adherence factor plasmid (pEAF), respectively. A/E is a histological lesion characterized by the destruction of microvilli and intimate bacterial adhesion to intestinal epithelial cells. The 35.6 Kb pathogenicity island known as Locus of Enterocyte Effacement (LEE) is responsible for the A/E lesion. In order to cause A/E, LEE comprises the structural components of the type III secretion system (TTSS). *E. coli* secreted protein A (EspA) is located on LEE4 operon, along with EspB, D and F. These enable the formation of a translocon, a needle like structure, which allows the injection of effector proteins into the host cell. **Objectives:** Cloning and expression of EspA protein from Enteropathogenic *Escherichia coli*. **Methods:** EspA gene was cloned into pGEM T-Easy and subcloned into pET 28a vector. **Results and Discussion:** Results were confirmed by PCR using primers based on the EspA sequence as previously described, and sequencing. Recombinant protein purification by chromatography affinity, antiserum production and immunologic assays are set as future goals for this study.

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12.12 Antibody production and cell populations in mice genetically selected for different antibody production

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Introduction: Selection III was developed with the aim of studying the genetic regulation of the adaptive immune response, producing lines with high (HIII) or low (LIII) secondary antibody response to *Salmonella* flagellar antigens. These mouse lines are characterized by a large difference in antibody titers, as well as the presence of multispecific effect that extends to complex antigens unrelated to the selection. These strains differ in their resistance to infection, susceptibility to autoimmune diseases and chemically-induced tumors. **Objective:** To analyze the capabilities of HIII and LIII mice to produce specific antibodies against a heterologous protein - bovine gamma globulin (BGG), investigating the behavior of these lines in both primary and secondary response, as well as the phenotypic profile of the cells activated in the spleen after immunization. **Methods:** Mice received two doses of BGG (0.5 mg/mL) in a 21- day interval. Blood samples were collected at 7, 14 and 21 days after the first and second immunizations. Specific IgM and IgG antibody levels were measured by ELISA. After 42 days, the phenotype of splenic and lymph node ($CD4^+$, $CD8^+$, $B220^+$) cells were characterized by flow cytometry. **Results and Discussion:** To date, our results demonstrated that, in both primary and secondary responses occurred an increase in antibody titers with similar kinetics in both lines. However, 7 days after the second immunization the females showed higher levels than males, which persisted until day 14. Male LIII mice showed the lowest levels of IgM, while female HIII mice showed the highest antibody secretion. No differences could be observed between High and Low mice in spleen cell populations. Thus, we conclude that the genetic factors related to high or low production of anti-*Salmonella* antibodies, inherited along the selection process of the HIII and LIII mouse lines, were specific and seem not to modulate the response against bovine gamma globulin.

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12.13 Standardization of cultures of murine megakaryocytes: influence of mouse strains and cultivation conditions.

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Introduction: Blood platelets are originated from extrusion of beaded projections, called proplatelets, from mature megakaryocytes (MK). In adult mice, MK are found in bone marrow, and are originated by expansion and differentiation of hematopoietic stem cells (HSC), promoted by thrombopoietin. During differentiation of MK, intracellular granules are formed, which are a rich source of growing factors (vascular endothelial growth factor, epithelial growth factor, platelet derived growth factor, transforming growth factor β), hemostatic factors (von Willebrand factor, fibrinogen, factors V and XIII), and adhesive proteins (P-selectin, fibronectin, vitronectin). Such factors modulate and accelerate wound healing processes, and may function as an alternative source of proteins for therapeutic topic treatment of wounds.**Objectives:** As a first step to obtain such proteins from MK, herein we evaluated conditions to cultivate MK from murine HSC. Furthermore, we evaluated which isogenic murine strains, C57Bl/6 and Balb/c, from the Animal House of Butantan Institute, provided higher yields of MKs in cultures. **Methods**: All experimental procedures were approved by CEUAIB (1001/2013). Adult male and female Balb/c and C57Bl/6 mice were euthanized by CO_2 inhalation, and then the bone marrow from femurs, tibias and humeri was removed. Mononuclear cells were cultivated in Dulbeccos's Modified Eagles's medium containing 10% fetal bovine serum, and cells were expanded and differentiated into megakaryocytes in the presence of 50 ng/mL murine thrombopoietin. **Results and Discussion**: Initial results showed that in bone marrow cells from both strains, after 3-4 days of incubation of thrombopoietin, few MK expanded and differentiated. MK are easily recognized in bone marrow cultures by their large size, and they are interspersed between smaller live and dead cells of other blood lineages. Cell viability was around 35% on day 03, and to 22% on day 04, showing that high cell density in cultures interfered with cell viability. Other conditions of cultivation of murine bone marrow cells, such as increasing the concentration of thrombopoietin in medium and decreasing cell density, are currently being tested.

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12.14 Routes of transplacentally transmission maternal-fetal of HPV

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Introduction: Human papillomavirus (HPV) is a member of the *Papillomaviridae* family that infects stratified epithelium of the skin and mucous membranes. It has circular double-stranded DNA genomes with size close to 8 kb. In spite of their small size, their molecular biology is very complex. Two oncogenes, E6 and E7, modulate the transformation process; two regulatory proteins, E1 and E2, modulate transcription and replication; and two structural proteins, L1 and L2, compose the viral capsid. HPV can be transmitted by sexual contact and mother to baby by delivery. HPV is classified according to its oncogenic potential. In most cases, HPV doesn't manifest symptoms and is spontaneously eliminated by the organism. However, of the 100 HPV different genotypes, 30 to 40 can affect both sex genital areas, causing several diseases like genital wart as well as cervix, vagina, vulva, anus, penis and cervical cancer. Furthermore it can cause tumor in the mouth and throat (oropharynx), benign manifesting as recurrent respiratory papillomatosis. Vertical transmission of HPV from mother to fetus is known to occur. Indeed, up to 80% of neonates born from women with genital HPV have HPV DNA detectable in their nasopharyngeal aspirate or oral mucosa, and this may persist for months or years. It is reported that, among infants who were positive for HPV-16 at birth, HPV-16 DNA could still be detected in 60% of infants at 6 months of age, and in other reports, HPV was noted to be persistent in the oral mucosa in 10% of 3 year old infants. **Objectives:**We intend to contribute with new data about possible alternative routes of neonates HPV infection, main responsible for the cervical cancer, detecting sequences of viral DNA in maternal blood cells, different placental parts, amniotic fluid and umbilical cord blood. This finding could suggest if maternal blood is a possible maternal-fetal route of HPV transmission. **Methods:** 135 Samples (maternal blood, umbilical cord blood, amniotic fluid and placenta) of HPV positive (69 samples) negative (66 samples) mothers will be collected in the operating room by someone capable of doing it. DNA will be extracted using Stratec molecular kit or Salting Out method, and the DNA quantity will be manually measured using nanodrop and afterwards PCR (Polymerase Chain Reaction) will be carried out in order to detect viral DNA sequences using MY09 and MY11 primers. **Results and Discussion:**Till now DNA of 3 maternal blood test samples were extracted, and 1,111.11 ng/µL of total DNA was the maximum obtained from the samples.

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12.15 Immunochemical and biological characterizationof*Naja annulifera* Peters (1854) snake venom

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Introduction: *Envenomation by poisoning animals* is a public health problem in rural areas of tropical and subtropical countries of Africa, Latin America, Asia and Oceania. Among the species of medical importance in the sub-Saharan Africa, *Naja annulifera*, family Elapidae, is involved in several accidents that may results in fatal respiratory arrest due to the presence of neurotoxins in its venom composition. Besides the severity of the accidents, the venom from *N. annulifera* was hardly characterized and the mechanisms by which it causes pathology remain poorly investigated. **Objectives:**The aim of the present study was to evaluate the toxic and pro-inflammatory properties of *N. annulifera*'s venom and its effect on the activation and regulation of the complement system. **Methods:**Venom from *N. annulifera* was tested to determinate its protein content, electrophoretic profile, sugar residues patterns and the presence of *cobra venom factor* (CVF). It was also tested the gelatinolytic, phospholipase and hyaluronidase activities of the venom and its ability to activate the complement system. In addition, BALB/c mice were injected with venomor saline, as negative control, to assess its lethal (LD_{50}) and edematogenic activities.**Results and Discussion:**The venom from *N. annulifera* contained 72.8% of proteins with a molecular weight varying from 13 to 174 kDa, including CVF, with some of the components presenting mannose and N-acetylglucosamine residues. The venom showed phospholipase and hyaluronidase activities but not gelatinolytic activities. In addition, the venom triggered the activation of the complement system by three pathways, alternative, classical and lectin. *N. annulifera'*svenom directly cleaved the alpha chain of the complement components C3 and C4, but not C5, generating anaphylatoxins and Terminal Complement Complex (TCC). However, the venom had no effect on the fluid phase, C1-Inh, neither on the membrane-bound, CD59, CR1 and DAF, complement regulatory proteins.Furthermore, the venom of *N. annulifera* induced a significant edematogenic activity and showed a LD_{50} of 94.14 µg in BALB/c mice inoculated in the left hind footpad and by intraperitoneal route, respectively.These data show the presence of toxic and proinflammatory components in the venom of *N. annulifera*, which in addition to proteases able to activate the complement system, may contribute to the development of clinical manifestations of envenomation.

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12.16 Standardization of analytical conditions of recombinant rabies virus glycoprotein from different sources

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Introduction: The rabies virus glycoprotein (RVGP) is the most important antigen for rabies prevention by vaccination strategies. The establishment of a recombinant vaccine against rabies has been the subject of many researches. A subunit vaccine bearing the RVGP as immunogen has to meet many requirements of safety and efficacy. Purity and immunogenicity are two of these requirements and can be achieved through efficient protocols of purification and analysis. **Objective:** To standardize the best analytical conditions of RVGP produced in different recombinant systems. **Methods:** Recombinant S2 cells (S2MtRVGP-His) were cultivated in T-flasks and RVGP expression was induced by adding $CuSO_4$. Adherent BHK-21 cells expressed RVGP after transduction with recombinant Semliki Forest Virus. Samples of cells expressing RVGP were resuspended with one of the four buffers: TD (25mM Tris-HCl, 137mM NaCl, 5mM KCl, 0.7mM Na_2PO_4), TM (50mM Tris-HCl, 500mM NaCl), T4 (25mM Tris-HCl, 25mM NaCl, 5mM $MgCl_2$) and TDM (25mM Tris-HCl, 65mM NaCl, 5mM $MgCl_2$, 0.7mM Na_2PO_4), each one in four different pH's (6.8, 7.2, 7.4 or 8.0) and two concentrations of DDM or IGEPAL (0.2 % or 0.04%), resulting in 64 formulations for cell lysis and RVGP solubilization. After incubation at 4 °C or room temperature for 30 or 60 minutes, samples were analyzed by Dot blotting, with rabbit anti-RVGP primary antibody, followed for a peroxidase conjugated mouse anti-rabbit antibody. Membranes were developed using the ECL technique. **Results and Discussion:** The best condition for RVGP solubilization from BHK-21 or S2 cells was achieved with buffers T4/DDM 0.04%/pH 6.8 and T4/DDM 0.2%/pH 6.8, both by incubation for 30 minutes at room temperature. The result was defined based on the dot area and intensity, comparatively among all situations tested. Data showed a combination of buffer, pH, incubation time and temperature that must be used for RVGP analysis. This standardization is of most interest, as performing RVGP analysis in one of these best conditions may provide reliable results and consequently to aid the establishment of a good purification protocol for RVGP.

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12.17 Identification of outer membrane proteins from one strain of atypicalenteropathogenic *E. coli* (EPEC)

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Introduction: Enteropathogenic *E. coli* has been identified as a main causative agent of acute diarrhea in developing country populations. Diarrhea is still one of the most significant cause of global child mortality between 0-5 years old. **Objective:** The goal of this study is to characterize and identify the outer membrane proteins (OMPs) of extracts derived from one strain of EPEC.**Methods:**Strain (9100-83) (serotype 0125:H6) was selected for this study. The OMPs were analyzed by two dimensional gel electrophoresis (2-DE): in the first dimension by focalization of 13cm, pH range of 4-7 strips (IPGphor III, GE Healthcare) and in the second by SDS-PAGE using 15% SDS-polyacrylamide gels (SE 600 Ruby, GE Healthcare). The identification was performed by removing the spots from the gels and digestion with trypsin followed by mass spectrometric analysis on ESI QTOF Ultima – Waters. The resulting data were analyzed with a non-redundant protein database (NCBInr) using Mascot v3.0 engine (Matrix Science). **Results and Discussion:** Seventy spots were identified, allowing for the characterization of thirty seven distinct proteins. Four proteins were OMPs or porins (Omp A, Omp W, Omp X and nucleoside channel receptor of phage T6 and colicin K) Two transporters were identified (ferrichrome outer membrane and fatty acid transporters). Twelve enzymes were detected (LysM/BON, phosphopentomutase, ribonucleoside hydrolase, manX, succinate dehydrogenase, kdpg aldolase, hydrolase-oxidase, ATP-dependent protease, transacetylase, phosphopyruvate hydratas and phosphoglycerate kinase). Moreover, one chaperonin (Groel GROES ADP7), one flagellin, five hypothetical proteins, one NAD-binding protein, two elongation factors, one protection protein (DPS), one inhibitor of C-type lysozyme (Ivy), one lipoprotein, two ribosomal proteins (50S) and two scaffolding proteins were identified. Omp A was one of the most abundant components. The results indicated that various proteins identified have important roles in membrane permeability and at least three of them, Omp A, Omp X and flagellin are involved in one of the first steps of pathogenicity, adhesion of the pathogen to host cells. The findings of proteins of unknown functions create opportunity to gather important missing information in future.

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12.18 Revision of the species of the genus *Neodiplothele* Mello-Leitão (Araneae, Mygalomorphae, Barychelidae)

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Introduction: The Barychelidae are represented in South America by Barychelinae, Trichopelmatinae and Sasoninae. The last subfamily is represented by three genera: *Cosmopelma* Simon, 1889, *Paracenobiopelma* Feio, 1952and *Neodiplothele* Mello-Leitão, 1917. The genus *Neodiplothele* is characterized by the presence of only two spinnerets, without cuspules on labium, few cuspules on the internal angle of the maxilla. *Neodiplothele* comprises only 4 species all from Brazil: *N. irregularis* Mello-Leitão, 1917 based on a female from Campina Grande, Paraíba; *N. fluminensis* Mello-Leitão, 1924 based on a male from Tijuca, Rio de Janeiro; *N. picta* Vellard, 1924, based on a female from Niterói, Rio de Janeiro and *N. leonardosi* Mello-Leitão, 1939 based on female from Paraguaçu, Bahia. In 1971, Bücherl and collaborators examined the holotypes of the two species: *N. fluminensis* and *N. leonardosi,* considering the last one an immature female. Raven transferred the genus to Nemesiidae after the examen of the holotypes from *N. irregularis* and *N. fluminensis*. Goloboff transferred the genus back to Barychelidae considering the wide clypeus, the reduced scopulae, the intercheliceral tumescence and the ridges on the bulb. **Objectives:** Revision of the species of the genus *Neodiplothele* and descriptions new species.**Methods:** The material examined is deposited in four Brazilian collections: Instituto Butantan, São Paulo (IBSP); Museu de Zoologia da Universidade de São Paulo, São Paulo (MZSP); Museu Nacional do Rio de Janeiro, Rio de Janeiro (MNRJ); Universidade Federal de Minas Gerais, Minas Gerais (UFMG). Female seminal receptacles were dissected and cleared in lactic acid for observation of internal structures. The photos were taken with a Leica DFC500 coupled to a Leica MZ16A and the same were mounted by the multifocal Leica Application Suite program (version 2.5.0). The drawings were made on a Leica MZ 12, with a camara lucida. **Results and Discussion:** Until now, the holotypes of the four known species could not be located. Based on the original descriptions and study of specimens near the type locality offer the possibility to redescribe *N. irregularis*, *N. picta*, *N. fluminensis* and *N. leonardosi*. After the examen of specimens of the collections five new species were identificated and described. The distribution range of all species are presented and increased for the states of Brazil: Bahia, Ceará, Espirito Santo, Goiás, Mato Grosso do Sul, Minas Gerais, Paraíba, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte, São Paulo, Santa Catarina, Sergipe e Tocantins.

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12.19 A new species of *Tmesiphantes* (Mygalomorphae, Theraphosidae) from the State of Pará, Brazil

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Introduction: Simonin1892 established *Tmesiphantes* based on a couple of specimens of *T. nubilus*, from the state of Bahia, Brazil. Yamamoto*et al.* in 2007 described three new species: *T.amadoi*, *T. caymmii* and *T. bethaniae*, also from Bahia. Two further species were added by Guadanucci & Silva (2012): *T. perp* and *T. riopretano*, both from the state of Minas Gerais, Brazil. Recently, Bertani *et al.* (2013) described *Tmesiphanteshypogeus* based on two females from two Brazilian caves from Bahia (Platnick, 2013). **Objectives:** Description of a new species of *Tmesiphantes* Simon, 1892,and a new distribution range for the genus. **Methods:** The material examined is deposited in the following institutions: Instituto Butantan, São Paulo (IBSP) and Faculdades Integradas do Tapajós, Pará (FIT). All measurements are in millimetres and were taken with a millimetric ocular lens. The total body length excludes chelicerae and spinnerets. The leg segment length was measured between the joints in dorsal view. The length and width of carapace, eye tubercle, labium and sternum are the maximum measured values. Female seminal receptacles were dissected and cleared in lactic acid for observation of internal structures. The photos were taken with a Leica DFC500 coupled to a Leica MZ16A and were processed with the multifocal Leica Application Suite program (version 2.5.0). **Results and Discussion:** During the identification of the mygalomorph spider's collection of the Faculdade Integradas do Tapajós, Belterra, Brazil a new species of the genus *Tmesiphantes* Simon was identified. This new species, *T. aridai* sp. nov., is the first species from this genus from the Amazonian region. The males of *Tmesiphantes aridai* sp. nov. differ from *T. perp* by the morphology of the palpal bulb without tegular basal projection, presenting a very slender embolus with shorter keels not extending until the tip. They also differ from *T. nubilus* by the tibial apophysis with two similarly sized branches the prolateral with a strong spine on the retrolateral margin. Females can be distinguished from *T. riopretano* by the two receptacles without constriction near the apex and differ from *T. caymmii* by the absence of a circular spot on the ventral side of its abdomen.

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12.20 Peritoneal exudate cytokine profile differs in "early" and "late" preclinical phase of pristane-induced arthritis.

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Introduction: HIII and LIII mice are extremely resistant or susceptible to pristane induced arthritis (PIA), and the early peritoneal cavity (PerC) response to pristane is crucial for disease outcome in this model. A very early (7d post injection) increase of IL12-p40 levels in peritoneal lavage fluid is observed in LIII mice, while inflammatory cytokines such as IL-1beta and IL-6 showed non-significant changes. Also, the peritoneal inflammatory infiltrate 7days post-pristane is distinct both quantitatively and qualitatively in these lines. It is not known if other cytokines have a role in this early or in later stages of the preclinical phase. **Objectives**: To characterize the peritoneal cytokines produced in the PerC of HIII and LIII mice and correlate the cytokine profiles to the type of infiltrated inflammatory cells in order to identify which cells are involved in susceptibility/resistance. **Methods**: Inbred HIII and LIII mice were single injected with 0,5 mL pristane i.p. and euthanized by CO2 exposure 7 or 35 days after injection. Control groups were not injected. After euthanasia, the peritoneal cavity (PerC) was washed with 3 mL non-supplemented RPMI1640 medium. The supernatant was frozen at -80ºC and the cell pellet suspended in 1 mL RPMI 1640 + 10% FBS. Total cells were counted in Malassez hemocytometer chambers. Cytokine ELISA (IL-1beta, IL-6, IL12p40, IFN-gamma, TNF-alpha) was carried out according to manufacturers' instructions. Results were analyzed by two-way ANOVA with Bonferroni post-tests. **Results and Discussion**: Total PerC cells increased two-fold ($p<0,001$) in pristane-treated LIII animals when compared to control mice 7 days post-pristane, returning to control levels at 35 days. Total cells in pristane treated HIII mice were similar to controls at all times. At 7 days post-pristane, IL12p40 was the only cytokine with significant changes in PerC, increasing in treated LIII but not in HIII mice. At 35 days, LIII mice had higher IFN-gamma while TNF-alpha and IL-6 levels increased only in HIII mice. Our results suggest that a polarization to a Th1-like peritoneal cytokine profile in LIII mice takes place during the preclinical phase of PIA and may be involved in the differential susceptibility of HIII/LIII lines.

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12.21 Occurrence of mortality in public Serpentarium of the Instituto Butantan between 2011-2013

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Introduction: Maintaining snakes in extensive and semiextensive captivity dates back to the early twentieth century with the purpose of producing biopharmaceuticals and public visitation. The geographical location and structure of captivity is essential to successful establishment and management of these animals. Inadequate conditions of captivity can facilitate the onset of a disease. The Serpentarium of Instituto Butantan is divided into three semiextensive captivity for snakes of the genus *Bothrops*, *Crotalus* and *Boa*. The captivity is composed of artificial shelters, shrub, trees, driftwood and a small stream. The substrate is composed of clayey soil covered with grass and small stones scattered. However, it has no artificial heating system, exposing the animals to natural climate variations. **Objectives:** To describe the mortality of the species maintained in captivity at the Serpentarium of Instituto Butantan and establish a relation to climatic conditions and data from clinical records. **Methods:** Forty-four clinical records of snakes of the genus *Bothrops*, *Crotalus* and *Boa* were evaluated and the data from death were compiled from January 2011 until July 2013. The average monthly air temperature and precipitation were obtained from IAG-USP. **Results and Discussion:** Over 2 years, 44 clinical records were evaluated: 36% of the deaths were recorded for *Bothrops* (n = 16), 16% for *Boa* (n = 07) and 48% (n = 21) for *Crotalus*. The highest incidence of deaths occurred from December to March - months related to higher temperatures and rainfall (summer). The genus *Crotalus* showed higher mortality, probably because this species inhabit open areas, rocky outcrops, from hot and dry climate and sandy soil typical of Cerrado. *Boaconstrictor* and *Bothrops jararaca* snakes are from dense forests with shrubby areas, humid climate and warmer temperatures and possibly for this reason show better survival due the structure and climatic variations which they are exposed to in the Serpentarium. Captive conditions, (clay soil and no heating) and exposure to climatic variations were factors that possibly contributed to the increased mortality in *Crotalus* during the summer season. Both factors may have contributed to higher humidity and cooling the captivity since the composition of the substrate does not allow adequate percolation of water, facilitating the spread of infectious diseases caused by fungi and bacteria. The data presented herein suggest that changes in substrate composition and the installation of a heating system are required to reduce mortality, promote wellness and prolong the life expectancy of *Crotalus*.

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12.22 Inhibition of E. coli O26 adhesion to human epithelial cells by antibodies against LPS

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Introduction: *E.coli* strains belonging to serogroup O26 are responsible for cases of infant death in developing countries and outbreaks of bloody diarrhea and hemolytic uremic syndrome in developed countries. One of the most effective alternatives to combat these pathogens is vaccination. However a vaccine against them has to generate antibodies capable of recognizing all *E.coli* strains belonging to serogroup O26 regardless the mechanism of virulence they present. It has been shown in the Laboratory of Bacteriology of the Butantan Institute that rabbit antibodies against O26 LPS were able to recognize different categories of *E.coli* belonging to this serogroup. However, for protection, these antibodies also need to inhibit bacterial adhesion to the intestinal epithelial cells. **Objectives:** The aim of this work was to verify the ability of rabbit antibodies against O26 LPS to inhibit the adhesion of different categories of O26 *E.coli* to human epithelial cells. **Methods:** HEp-2 cells were incubated with atypicalenteropathogenic *E.coli* (aEPEC) and enterohemorragic *E.coli* (EHEC) belonging to serogroup O26 in the presence or absence of rabbit serum against O26 LPS. After incubation cells were fixed with methanol and stained with methylene blue for light microscopy visualization. **Results and Discussion**: The results showed that the antibodies were able in a dose dependent manner to inhibit the adhesion to epithelial cells of EHEC and aEPEC belonging to O26 serogroup. In summary, the results of this study indicate that detoxified O26 LPS is a good candidate of antigen to be used in a vaccine formulation against different categories of O26 *E.coli.*

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12.23 Erythrolamprus miliaris orinus Cope, 1868 (Serpentes: Dipsadidae: Xenodontinae): Melanism

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Introduction: Melanism is a chromatic phenomenon caused by excessive concentration of melanin in the skin, resulting in animals with a phenotype darker than usual. The incidence of melanism has been reported for different classes of vertebrates, including reptiles, and is most frequently observed in specimens from high latitudes or elevation. Considering Serpentes, melanism was already documented in a couple families - e.g. Colubridae and Viperidae. The genus Erythrolamprus Boie, 1826 currently comprises 42 species widely distributed from Honduras south to Argentina. Populations of Erythrolamprus miliaris orinus Cope, 1868 from Atlantic forest (specifically from the coastal area of the state of São Paulo, southeastern Brazil) exhibit a conspicuous color pattern: with dorsal scales showing black borders and yellow centers, and belly creamish white. **Objectives**: The present study reports a case of melanism in Erythrolamprus miliaris orinus. The specimen was deposited in the herpetological collection of Butantan Institute "Alphonse Richard Hoge" (IBSP 81992) and comes from Bertioga (23° 85' S 46° 13' W), a coastal city in the state of São Paulo. **Methods**: This specimen is an immature male of 220 mm snout-vent length and 59 mm caudal length. Meristic data showed 17/17/15 dorsal scales rows, 161 ventral and 60 subcaudal scales. The specimen have a black coloration throughout the body, except for small pale spots on gular region up to the level of fifteenth ventral scale. **Results and Discussion**: Melanism is unusual for most species of Erythrolamprus. Chromatic phenomena reported for this genus are frequently related to mimicry, albinism, and leucism. Therefore, as far as we know, this represents the first report of melanism in Erytrholamprus. Melanic condition in Iberian snakes is relatively frequent, mainly in mountainous areas with low solar incidence. Studies about populations of Vipera seoanei in the Cantabrian mountain range (northern Spain) show 38% of melanic specimens. However, records of this phenomenon for Neotropical snakes are not common. Therefore, we hope this report enables further investigations on the environmental costs associated to melanism in tropical snakes.

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12.24 A new lysozyme found in the hemolymph from pupae of *Lonomia obliqua*

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Introduction: Insects represent 55% of the Earth's biodiversity and 85% of all animals. They are found in all regions, from the poles to the deserts, and this wide distribution has stimulated the search of new therapeutic agents in the class arthropoda. *L. obliqua* is an insect with medical importance, due to larval phase, that has urticant setae with toxin that causes hemorrhagic effects. Several components have been isolated and characterized in the venom of *L. obliqua*, such as Losac, an activator of factor X of the coagulation cascade, and Lopap, a prothrombin activator. In the hemolymph there is already been identified some bioactive molecules with fibrinogenolytic, antiviral, antimicrobial and antiapoptotic activity. **Objectives:** The objective of this study is to purify and characterize bioactive molecules present in the hemolymph from pupae of *L. obliqua.* **Methods:**21 pupae were maintained in control group and 41 pupae were challenged with Gram negative bacteria *E. coli* and Gram positive bacteria *M. luteus.* The hemolymph of the two groups were collected after 48 hours and submitted to acid extraction with 2M acetic acid, the samples were centrifuged and the soluble part was pre-purified with classic Sep–Pak C18 cartridges, being eluted with acetonitrile 40% and 80%. The obtained fractions were concentrated in a vacuum centrifuge. The fraction 40% was reconstituted with trifluoroacetic acid 0.05% and was applied to reverse phase chromatography on a semi preparative Jupiter C18 column. Elution was performed with a linear gradient of 2% to 60% of acetonitrile in acidified water over 60 min at a flow rate of 1.5 mL/min. The samples obtained were evaluated for presence of antibacterial activity by a liquid growth inhibition assay against *Micrococcus luteus* A270. Fractions that showed antibacterial activity were analyzed by electrophoresis, mass spectrometry and “de novo” sequencing. **Results and Discussion:** 9 fractions showed antibacterial activity against *M. luteus* A270. One fraction was selected and their analysisindicated similarity with a type of lysozyme found in moth *Hyalophora cecropia.* By electrophoresis, one band was identified on the height 14 kDa, corroborating the data presented in literature, that shows lysozyme of the moth *H. cecropia* has 13.8 kDa. Lysozymes are an important class of enzymes found in insects, that hydrolise the β-1,4-glycosidic bond between the N-acetylmuramic acid and N-acetylglucosamine residues in the glycan portion of peptidoglycan, and this hydrolysis results in bacterial cell lysis. The other fractions withantibacterial activity are being analyzed and characterized.

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12.25 Through the looking glass: the spectacle in gymnophthalmid lizards

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Introduction: The anatomy and development of the eyelids in squamate reptiles are still relatively understudied, considering its variation within the group. The neotropical Gymnophthalmini are traditionally characterized by having lost the eyelids, but such structure was never studied in detail. **Objectives:** Here we present a comparative study of the embryology and adult anatomy and histology of some eye's adnexa in gymnophthalmid lizards and establish a hypothesis for the homology and evolution of the eyelids and of the spectacle in the tribe Gymnophthalmini. **Methods:** We analyzed the embryonic development and the adult morphology of the eye in the mayor clades of the lizard family Gymnophthalmidae, with special focus to the eyelids, the nictitating membrane and the spectacle. Specimens of *Alopoglossus angulatus*, *Tretioscincus agilis*, *Tretioscincus oriximinensis*, *Micrablepharus maximiliani*, *Nothobachia ablephara* and *Calyptommatus sinebrachiatus* were obtained from the herpetological collection of the MZUSP. For histology, the heads were decalcified, embedded in historesin and transversally serial sectioned. For scanning electron microscopy, the heads were sagitally divided in two halves dried in a critical point device and covered with gold. **Results and Discussion:** We show that the eyes in some Gymnophthalmini are covered by a spectacle, formed by the embryonic fusion of the dorsal and ventral eyelids. The embryonic fusion of the eyelids in Gymnophthalmini is a possible synapomorphic character of the tribe. The genus *Tretioscincus*, which floats either as sister to all other Gymnophthalmini, or nested within the group is unique in showing functional and movable eyelids. Thus, the presence of functional eyelids can be either considered as the primitive condition for the Gymnophthalmini or as a re-acquisition of the character, showing the importance of a well-established phylogenetic hypothesis for understanding morphological evolution.

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12.26 Revision of the Neotropical spider genus *Xiruana* Brescovit 1996 (Araneae: Anyphaeninae, Anyphaenidae)

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Introduction: The family Anyphaenidae is divided into three subfamilies: Malenellinae, Amaurobioidinae and Anyphaeninae. These spiders are known as ghost spiders due to their rapid movement on the bushes, presents small to medium size with an entire body length of 2.5 to 14 mm and generally, inhabiting environments such as forests, deserts and even semi-arid regions. Anyphaeninae present at moment 33 genera in the Neotropical region. Among them the genus *Xiruana* Brescovit (1997) was chosen for a revision in the PAP project. This genus present four species: the type species *X. gracilipes* from Brazil and Argentina, *X. affinis* and *X. hirsuta*, both from Brazil and *X. tetraseta* from Paraguay. The genus is distinguished from others in Anyphaeninae by the combination of the following characters: tracheal spiracle almost near the epigastric furrow, palp of the males with bifid ATR, conductor with a median groove and epigynum of females with large median transversal septum and concave and sclerotized lateral borders. **Objectives**: To revise the Neotropical species of the genus *Xiruana*, determine and describe possible new species, elaborate a dichotomous key for species and mapping the geographical distribution from the genus. **Methods:** The specimens of the genus were examined through loans from national and foreign collections, but initially we worked with the material deposited at the Butantan Institute, São Paulo (IBSP). For observation of the male genitalia the following procedure was performed: the palp was expanded through immersion in a concentrated solution of KOH (10%) and subsequently placed in distilled water and female genitalia examined after digestion with a hot solution of KOH (10-20%). All measurements were done in millimeters (mm). The terminology and morphologies were expressed according with the pattern used in Anyphaenidae. **Results and Discussion:** A total of 184 specimens were analysed from the material of the South and Southeast of Brazil, Argentina, Bolivia, Peru and Uruguay. This preliminary analysis of the material revealed three undescribed species, with males and females, one from Ribeirão Preto and other from Rio Claro, in the state of São Paulo and the third from São Miguel do Oeste, in the state of Santa Catarina. These species will be described and the cast now to be worked have seven species, all from Neotropical region.

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12.27 Enzymatic characterization and antigenic cross-reactivity of *Tityus neglectus* scorpion venom

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Introduction: Scorpionism is considered a public health problem in Brazil. Bites in humans caused by genus *Tityus* are characterized mainly by intense pain, neurotoxic effects and can lead to death by pulmonary edema. Most species are found in tropical and subtropical environments. The genus *Tityus* have great ecological elasticity and can be found in different environments, including those modified by humans. To date, the species responsible for the most serious accidents in Brazil are: *T. serrulatus*, *T. stigmurus*, *T. bahiensis* and *T. obscurus*. However, 57 different species of genus *Tityus* have been described in Brazil. The *Tityus neglectus* scorpions are found in northeastern of Brazil, mainly in the base of bromeliads. This scorpion have size up to 78 mm, a yellow color brown ranging to reddish brown and the last two caudal segments and telson are black. So far there are no reports of envenomation with *T. neglectus*. **Objectives:** Characterization of biochemical and immunochemical properties of *T. neglectus* venom comparing with *T. serrulatus* and *T. stigmurus* venoms.**Methods:** Silver stained SDS-PAGE (12%) was used to compare the protein profile of *T. neglectus, T. serrulatus* and *T. stigmurus* venoms (20 µg).Zymography was used to detect proteolytic and hyaluronidase activities in venoms (100 µg) using fibrinogen (0.5 mg/mL), casein (2 mg/mL), gelatin (2 mg/mL) or hyaluronic acid (170 µg/mL) as substrate. Antigenic cross-reactivity using antiarachnid serum (AAS) and antiscorpionic serum (ASS) was detected by ELISA and Western Blotting. **Results and Discussion:** Many components with similar molecular masses between 50-37 kDa and 25-10 kDa were observed in all venoms. However some bands around 150-100 kDa and 15-10 kDa regions were observed exclusively in *T. neglectus.* Only hyaluronidase activity was observed using 100 µg in allvenoms (bands around 50-37 kDa). Cross-reactivity among venoms was detected, and significant differences on titers were noticed between *T. neglectus* (64,000), *T. serrulatus* and *T. stigmurus* titer (512,000). By Western Blotting it was observed that some components of the three venoms were recognized by AAS and ASS. However, bands around 15-10 kDa of *T. neglectus* venom were not recognized by both antisera. Our results demonstrated that *T. neglectus* venom has different components when compared with *T. serrulatus* and *T. stigmurus* venoms, which can influence the toxic activity of *T. neglectus* venom.

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12.28 Inflammatory response induced by phospholipases A2 isolated from *Micrurus lemniscatus* venom on hippocampal cell culture.

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Introduction: *Micrurus* is the most representative genus as far as abundance and diversity are concerned, with a great number of species found in South America and Southern United States. Previously we showed that the phospholipases A_2 (PLA_2s) neurotoxins, MlTx-8 and MlTx-9, isolated from *M. lemniscatus* venom reduce the trans membrane mitochondrial potential and increase the intracellular calcium concentration. Inspection using fluorescent images and ultrastructural analysis by scanning and transmission electron microscopy showed that multiphase injury is characterized by overlapping cell death phenotypes. Shrinkage, membrane blebbing, chromatin condensation, nucleosomal DNA fragmentation and the formation of apoptotic bodies were observed. **Objectives:** The present work was designed to investigate an inflammatory response induced by these PLA_2s-neurotoxins. For this purpose, we determined the level of cytokines and chemokines in culture supernatant incubated with MlTx-8 or MlTx-9. The excytotoxicity was also evaluated through the glutamate levels in the hippocampal cells incubated for 30 min with the toxins. **Methods:** Hippocampus of E18-E19 Wistar rats fetuses were dissociated. This protocol was approved by the Ethics Committee of the Butantan Institute (nº992/12). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2. On the seventh day, cells were incubated with MlTx-8 or MlTx-9 (0.74 and 7.4 nM) for 24 hours or 30 min, depending on the experiment. The cytokines and chemokines were determined through the Luminex xMAP method in 50μL of the culture supernatant and quantified by cytometry. TNF-α concentration was assessed in culture supernatant by cytotoxicity assay using the fibroblast continuous cell line L_{929}. Statistical analysis was performed by one-way ANOVA followed by Tukey's posttest ($p \leq 0.05$). **Results and Discussion:** Incubation of the hippocampal cell cultures with MlTx-8 or MlTx-9 (0.74 and 7.4 nM) for 30 min was not able to alter the glutamate release. Both PLA_2s-neurotoxins increased significantly the IL-1β, TNF-α and MIP-1 levels. MlTx-8 and MlTx-9 were not able to alter the IL-6, IL-10 and RANTES release. The IFN-□ levels were not detected by the method employed. Our results suggest the presence of an inflammatory response induced by the PLA_2s-neurotoxins showed by the increased of pro-inflammatory cytokines and chemokines.

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12.29 Antimicrobial resistance profile and pellicle formation on glass by *Escherichia coli* diarrheagenic strains

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Introduction: There are six pathotypes of diarrheagenic *E. coli* (DEC): Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative (EAEC), Enteroinvasive *E. coli* (EIEC); Shiga toxin-producing *E. coli* (STEC), and Diffusely adherent *E. coli* (DAEC), which differ in their pathogenesis and clinical syndromes caused. The bacteria can adhere to surfaces, surrounded by a matrix of exopolyssaccharides forming biofilms, which favors bacterial persistence and resistance against antibiotics. **Objectives:** The aim of this study was to evaluate the antimicrobial resistance profile of 30 DEC strains and to investigate the ability of pellicle formation in glass tubes. **Methods:** We analyzed 30 DEC strains (atypical EPEC - 11, ETEC – 10, EAEC - 05, STEC - 02, EIEC – 01, and typical EPEC - 01), isolated from cases of childhood diarrhea in Salvador, Bahia. The antimicrobial susceptibility tests were performed according to the method of disk diffusion (Kirby and Bauer) and the following antibiotics: Amoxicillin (AMO), Amoxicillin + Clavulanate (AMC), Cefoxitin (CFO), Cephalothin (CEP), Ceftriaxone (CRO), Aztreonam (ATM), Imipenem (IPM), Meropenem (MER), Amikacin (AMI), Gentamicin (GEN), Streptomycin (EST), Ciprofloxacin (CIP), Tetracycline (TET), Sulfametoxazol + Trimethoprim (SUT), and Sulfonamide (SUL). The *E. coli* strains were assayed to pellicles formation in glass tubes, constituting biofilm, in LB broth at 26°C and 37°C for 72 hours, with and without agitation at 210 rpm. **Results and Discussion:** The DEC strains showed 100% sensitivity to AMI, ATM, IPM, CFO, GEN, CIP, CRO and MER. The aEPEC strains showed resistance to AMO, SUT, SUL, EST, TET (18 to 45%). The ETEC strains presented resistance to SUT, SUL, AMO, EST and CEP (10 to 50%), while the EAEC strains were resistant to TET, SUT, SUL, AMO, EST and CEP (20 to 80%). The STEC strain presented intermediary sensitivity to SUT and CEP. The EIEC strain was sensitive to all antibiotics tested. The tEPEC strain showed multidrug-resistance, as well the EAEC (80%), ETEC (50%) and aEPEC (45.5%) strains. 20 to 30%of ETEC and EAEC strains incubated at 26° C and 37° C formed pellicle, except for 60% of EAEC strains at 37° C, which formed more biofilm. The pellicle formation was observed mainly in static growth at 37° C in 26.7% of the strains, whereas 6.7% of strains at 26° C. Despite the high antimicrobial resistance of the DEC strains, we found several drugs that can be used as an alternative in the cases that the use of drugs is necessary. The EAEC strains showed higher levels of resistance to antimicrobials and a higher pellicle formation, which indicates its ability to form biofilms and possibly larger antimicrobial resistance, and should be better monitored.

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12.30 Characterization of algogenic compounds found in rat platelet releasate.

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Introduction: Platelets are essential cells to the maintenance of hemostasis, and when activated they release several compounds from their granules. Recently, platelets have also been characterized as inflammatory cells, because they have a high phlogistic and algogenic potential, contributing to the development of inflammation and pain. In a previous report, weshowed that whole platelets or platelet releasate (PR) from platelet granules when injected intraplantarly (i.pl.) in rats caused an increased pain threshold. Thus, identification of algogenic compounds secreted during platelet activation is important for understanding the role of platelets in the pathogenesis of pain, as well to seek for new analgesics. **Objectives:** To characterize the compound(s) found in PR that accounts for evoking hyperalgesia in rats. **Methods**: All of the procedures using animals were approved by CEUAIB (980/2012). Male Wistar rats (160-180g) were used to obtain PR (800 x 10^9 platelets/L). In order to evaluate hyperalgesia, rats were submitted to the paw pressure test in hind paws at 1, 2 and 4 h after i.pl. injection (100 µL/paw) of samples. To evaluate the molecular mass of these compounds, PR aliquots were centrifuged in filter units with 3 kDa nominal molecular weight cut-off (Millipore) at 3220 *g* for 20 min. Stability of ultrafiltered samples was tested by either freezing at -80°C, or freezing and lyophilization for 2 days. **Results and Discussion:** At the day of experiments, the samples were ultrafiltered, and two fractions were obtained: the supernatant, constituted of compounds with molecular mass superior to 3kDa, and the ultrafiltered sample, containing compounds with molecular mass lower than 3kDa. Those fractions were evaluated if they exhibited algogenic activity, and compared to the positive control (PR). Ultrafiltered samples evoked hyperalgesic response up to 4h, similarly to the positive control (PR). However, the supernatant failed to induce hyperalgesia or analgesia in rats. These data demonstrate that compounds with molecular mass less than 3 kDa account for the algogenic activity of platelets. When the ultrafiltered samples were frozen, or frozen and lyophilized, no response was noticed in comparison to the initial measure, demonstrating that freezing *per se* destroyed the hyperalgesic activity. Thus, we conclude that algogenic compounds found in PR have molecular mass less than 3kDa and lose their ability to induce hyperalgesia in rats by freezing or lyophilization. Currently, these compounds are being purified and characterized by HPLC.

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12.31 I-Alternatives of soybean peptone for the culture of *Haemophilus influenzae* type b: soybean peptone derivatives and yeast extract

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Introduction: *Haemophilus influenzae* type b (Hib) is a facultative anaerobic, Gram-negative, coccobacillusbacterium and requires blood factors as hemin and NAD for its growth. Hib is the most prevalent serotype of clinical importance and meningitis is the most serious form of infection among children bellow 2 years old, elderly and immunodeficient. Polysaccharide composed by polyribosylribitol phosphate (PRP) is the main factor of virulence and the purified PRP is the base of Hib vaccine. Studies based on Hib's metabolism as well as to know the components of medium culture should contribute for the improvement of PRP production and may reduce the final production cost. Culture medium for Hibuse soybean peptone which has a 30% of carbohydrates some of them difficult to metabolize and it is interesting to look for alternatives. **Objectives:** To study different substitutes of soybean peptone (Soytone™) for Hib growth and evaluate the PRP production. **Methods:** 1)Soytone with concentrations of 10 g/L, 30 g/L and 50 g/L were submitted to tangential ultrafiltration 5kDa and each fraction ultrafiltrate (UF) and concentrate (C5k) was used to compose the medium, separately; 2) Yeast Extract (YE) was used with concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L without Soytone. 3) Medium MP with soytone and without YE. The MP medium was used as control, with Soytone and YE, in all essays. Each medium, 100 mL, were distributed in 500 mL flasks and cultivation was done at 37°C and orbital agitation 250 rpm. Samples were collected each hour to follow the cell growth by reading DO_{540nm} and PRP was measured after 8 hour of cultivation by Bial method. **Results and Discussion:** 1) the control medium showed a specific growth (μ_{max} = 0.57 h^{-1}), an OD_{540nm}= 5.47 and an exponential growth of 6.5 h. Soytone UF fraction at 50 g/L showed the highest μ_{max} = 0.74 h^{-1} with short exponential growth 3.2 h and low OD_{540nm} of 0.95 and PRP = 52.2 mg/L. The UF of 10 and 30 g/L Soytone had a μ_{max} = 0.50~0.55 h^{-1}, vigorous growth for 8.3~5.3 h^{-1} and OD_{540nm} 5.13~4.19. This results showed that the higher Soytone concentration resulted in higher μ_{max}, shorter time of exponential growth and low OD_{540nm}. Regarding to the C5k fraction, the values of μ_{max} were around 0.59 h^{-1} in the cultivation interval of 8.3 h; OD_{540nm} were similar among them and the value of PRP was 200 mg/L. The concentrate of Soytone keeps most of the nutrients of whole Soytone but the UF lost some nutrients 3) Soytone alone with 10 g/L cannot substitute YE, Soytone achieved μ_{max} of 0.42 h^{-1} in 8.0 h and OD_{540nm} 3.7. The result indicates that only the YE could be used in substitution of Soytone.

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12.32 II-Alternatives of soybean peptone for the culture of *Haemophilus influenzae* type b: soy protein concentrate, molasses and malt extract

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Introduction: *Haemophilus influenzae* type b (Hib) is a Gram negative capsulated bacterium, microaerophilic, coccobacillusshape and requires blood factors for its growth. Pneumonia and meningitis are the most important invasive diseases related to children of age-group bellow 2 years old, elderly and immunodeficient. Capsular polysaccharide is the main virulence factor and is used as component of the vaccine against Hib. Culture media for vaccine production used to get rid of components from animal origin such as meat/casein peptones and the regulatory authorities recommend replace them by protein peptones from vegetal origin specially soybean peptones (Soytone™). Studies about Hib's metabolism and medium composition are important tools for improving the production of PRP in order to manufacture vaccine. Brazil is the major producer and processor of soybean to obtain oil, soy flour, soy protein concentrate (SPC), and so on, however it does not produce soy protein isolate and soy peptones. **Objectives:** Toreplace soy peptone by SPC, Soybean Molasses (SM) or Malt Extract (ME) for the Hib cultivation in the MP complex medium. **Methods:** All assays were performed in 500 mL flasks containing 100 mL of MP complex medium with or SPCor/and SM orME, at 250 rpm and 37 °C. SPC and SM were autoclaved and insoluble removed by centrifugation and incorporated in the medium MP separately or together. ME sterilized by filtration and tested in the MP medium replacing Soytone.The original MP medium with Soytone and yeast extract was used as control. **Results and Discussion:** The media containing SPC or SPC+SM showed the same maximum specific growth rate (μ_{max}) similar to the control (0.61 h^{-1} vs. 0.57 h^{-1}) and the maximum OD_{540nm} was 5.4. The μ_{max} obtained by the medium SM alone was 0.53 h^{-1} and OD_{540nm} of 4.1. In the ME medium μ_{max} was 0.42 h^{-1} and a long duration of exponential growth (9.2 h) with an $OD_{540nm}$of 5.43 was observed. ME is composed mostly of carbohydrates and the nitrogen content is 1/30 of Soytone with slow growth but it reach near the final bacteria concentration of control (OD_{540nm} 5.15 vs. 5.4). The SPC could be a substitution of soybean peptones.

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12.33 Influence of recombinant S2 cell population enrichment on rabies virus glycoprotein expression and especific RNA and DNAquantities.

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Introduction: The maintenance of selective pressure during all the cultivation time or population enrichment of *Drosophila melanogaster* (S2) cells expressing recombinant rabies virus glycoprotein (RVGP) are two methods which can increase the RVGP productivity. **Objectives:** To demonstrate the differences between the quantities of RVGPmRNA and RVGP-DNA in enriched S2 recombinant cell lines, submitted to cycloheximide (CHX) treatment. **Methods:** S2MtRVGPHy cell population (untreated control) generated other three populations: S2MtRVGPHy+Hy, obtained after selective pressure using hygromycin for 2 weeks and both S2MtRVGPHy-M2 and S2MtRVGPHy-M3, obtained after immunomagnetic enrichment (MACS, Miltenyl Biotec) of expressing cells, using rabbit polyclonal and mouse monoclonal antibodies, respectively. For studying expression profiles, an inhibitor of translation in eukaryotes (CHX) was added to cultures for translation blockage. Cell populations were induced with $CuSO_4$ for RVGP expression and submitted to CHX treatment for translation interruption. Samples were taken in different time points and analyzed by ELISA, qPCR and qRT-PCR. **Results and Discussion:** All cell populations presented small differences in RVGP-DNA content as analysed by qPCR. Before treating the cultures with CHX, cell populations showed very similar amounts of RVGPmRNA and were expressing RVGP at concentrations of 23.1 (S2MtRVGPHy), 31.9 (S2MtRVGPHy-M2), 65.3 (S2MtRVGPHy-M3) and 45.7 ng/10^6 cells (S2MtRVGPHy+Hy), showing that both strategies were successful on improving RVGP expression. When CHX was added to cultures, the amounts of RVGP decreased probably due to protein degradation and translation interruption. As expected, RVGPmRNA levels increased. As these methods not statistically changed the amount of RVGP-DNA copies/cell between the cell populations in study, the differences in RVGP expression could be attributed to different transcription and translation rates. The amounts of accumulated RVGPmRNA showed that cell populations exhibited different profiles of transcription and translation for glycoprotein expression. While S2MtRVGPHy-M3 produced the highest level of RVGP (65.3 ng/10^6 cells), it showed the smallest level of RVGPmRNA accumulation (R = 5.7) among all populations. As S2MtRVGPHy+Hy produced the second highest level of RVGP (45.7 ng/10^6 cells), and showed the highest RVGPmRNA accumulation level (R = 499.4), probably cells present different rates of translating RNA due mainly to metabolic differences. Future experiments undergoing more broadly kinetic evaluations of RVGP and RVGPmRNA may contribute to better understand these results.

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12.34 Cytotoxic effect of the crude venom of *Bothrops jararacussu* on mononuclear leukocytes from human peripheral blood

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Introduction: Snake envenomation is a public health problem, considered as a neglected tropical condition by the World Health Organization. In Brazil, the *Bothrops* genus is responsible for 87% of ophidian accidents, causing local effects such as hemorrhage, edema, pain and necrosis, and less frequent systemic effects, as hematuria and shock. Particularly, shock may be related to activation of the complement system by *Bothrops* venoms, once it has been demonstrated the anticomplementar activity of venoms from 19 species of *Bothrops* found in Brazil. Among these, the venom of *Bothrops jararacussu*, one of the important medical species, poorly activated the complement classical pathway and did not activate the alternative and lectin pathways, suggesting that there may be other mechanisms for shock induction by this venom, such as direct action on peripheral blood leukocytes. **Objectives:** To investigate the cytotoxic effect of the crude venom of *B. jararacussu* on mononuclear leukocytes from human peripheral blood. **Methods:** Lymphocytes and monocytes were obtained from peripheral blood of healthy donors, by density gradient separation, using Ficoll-Hypaque 1077, followed by monocytes adhesion for 24 hours. Cells were incubated, separately, with different venom concentrations during 24 hours, and cell viability was analyzed by MTT assay. Two cellular concentrations (5×10^5/well e 2.5×10^5/well) were used. The supernatants from some monocytes cultures were collected to determine the concentration of nitric oxide (NO) by Griess reaction. **Results and Discussion:** Using the cellular concentration of 5×10^5/well, lymphocytes viability was not significantly affected by any venom concentration, compared to the control, although there seemed to be a tendency of decreased viability with 50 µg/mL and 100 µg/mL of the venom. Monocytes showed no change in viability. On the other hand, using the cellular concentration of 2.5×10^5/well, there was a significant decrease in lymphocytes viability in cultures incubated with 50 µg/mL and 100 µg/mL of the venom, in which the percentage of viability decreased to 76 % and 60 %, respectively. Monocytes viability was affected only by the concentration of 100 µg/mL, decreasing to 71% of viability. Low concentrations of NO were found in these monocytes cultures. Our results suggest that *B. jararacussu* crude venom can affect the viability of mononuclear leukocytes from human peripheral blood. Further studies are being performed to investigate the role of this effect in human envenomation.

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12.35 *Bothrops jararacussu* (jararacussu). Ophiophagy.

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Introduction: *Bothrops jararacussu* is a terrestrial snake frequently found throughout the Atlantic forest domain along streams. The genus *Bothrops* is characterized by a generalist diet and ontogenetic shifts from ectothermic to endothermic prey. Previous studies indicate that *B.jararacussu* is mainly nocturnal and prey small mammals, although young individuals may hunt during the day and consume ectothermic prey. On 19 april 2013 at 2210 h, during fieldwork of herpetological community study, we collected a young male of *Bothrops jararacussu* (MRCM0604, 340 mm SVL, 52 mm TL) on the road (primary forest border) in Sete Barras municipality, São Paulo, southeastern Brazil (24.31°S, 48.11°W, datum WGS84), that contained an intact *Sordellina punctata* (Dotted Brown Snake) (333 mm SVL, 103 mm TL) ingested by head first. **Objectives:** Notify ophiophagy this rare case of the species *Bothrops jararacussu*, who ingested a snake species *Sordelina punctata*. **Methods:** The specimen was collected in *Bothrops jararacussu* road (border of primary forest) in the municipality of Sete Barras, São Paulo, southeastern Brazil (24.31 ° S, 48.11 ° W, WGS84 datum), with the permission of Mr Matias Mignon Mickenhagen to access field sites. Posthumously euthanized with anesthetic thiopental sodium. After measuring. After it was fixed with 10% formalin, and stored in 70% alcohol. In the laboratory the specimen was opened by ventral scales with scissors, and it was found that had the stomach contents, a snake species *Sordellina punctata* (Dotted Brown Snake). **Results and Discussion:** Although viperideos possess skills to subdue preysmuch larger than themselves, the prey of this snake was longer than its predator. Another similar case was described by Marques O. et al 2004, where he collected a *Bothrops jararacussu* who had ingested one *Chironius bicarinattus* greater than its length . The snake ingested in this case was swallowed up by the head, and was almost intact in its predator. The prey had measures of length and mass relatively larger than its predator.

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12.36 Characterization of a novel putative leptospiral outer membrane protein.

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Introduction: Leptospirosis is considered the most spread zoonosis in the world, caused by pathogenic bacteria of the genus *Leptospira*. In developing countries, due to the poor sanitation, it constitutes a serious public health problem. The disease outbreaks occur during the rainy seasons, when the floods are usual, which contributes to the dissemination of the leptospirosis. The carriers may be wild or domestic animals, especially rodents. The symptoms of the disease vary from flu-like to a potentially fatal disease with multiorgan involvement. Currently, there is no efficient vaccine or diagnostic kit for human leptospirosis. Based on the genomic sequence of *L. interrogans* sorovar Copenhageni, the main objective of our research group is to search for potential proteins that can be used as a vaccine or diagnostic kit. **Objectives:** This work aims to select, clone, express, purify and characterize a predicted outer membrane protein identified in the *L. interrogans* genome. **Methods:** The chosengene LIC10821 was amplified from leptospiral genomic DNA and cloned into pAE expression vector. Protein expression was induced by addition of IPTG in culture of transformed *E. coli* BL21 (DE3) C43 bacteria. The recombinant protein was purified by metal affinity chromatography and eluted with imidazole. The purified protein was inoculated into Balb/C mice in order to obtain hyper immune sera. Circular dichroism of the recombinant protein rLIC10821 was performed to define the secondary structure content. Sera from patients diagnosed with leptospirosis were tested for reactivity against the recombinant protein. **Results and discussion:** The recombinant protein was expressed as inclusion bodies and refolded successfully by urea removal. Circular dichroism of rLIC10821 indicated the correct fold of the protein, according to bioinformatics prediction, showing predominantly beta sheets. ELISA from hyper immune serum showed titers of 8000. The low reactivity against sera from positive leptospirosis serum samples (15% MAT-) (11% MAT+) suggests that this protein is not a candidate for diagnosis of the disease. In conclusion, the leptospiral recombinant protein rLIC10821 was efficiently obtained in *E. coli*, and its protective immune activity will be assayed in animal model in order to evaluate its potential as a subunit vaccine against leptospirosis.

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12.37 Insularin (GST-INS) inhibit adhesion of endothelial cells to platelets and induces HUVEC detachment

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Introduction: Disintegrins are RGD peptides that bind specifically to integrins αIIbβ3, α5β1 and αvβ3 expressed on platelets and other cells including vascular endothelial cells and some tumor cells. Depending on the type of tumor, various aspects of cancer progression may be affected by platelets, including tumor angiogenesis and metastasis and these phenomena are often dependent on the interaction between αIIbβ3 and αvβ3. Thus, an interaction between both integrins is a relevant mechanism involved in adhesion events between platelets, endothelial and some tumor cells. It has been shown that disintegrins inhibited tumor-associated angiogenesis. Some studies showed that disintegrins primarily affects the newly forming vessels but not preexisting large vessels. However, only a few reports have investigated the effects of disintegrins on cell detachment. Insularin (GST-INS) is a recombinant disintegrin from *Bothrops insularis* venom that inhibits platelet aggregation ADP-induced and inhibits endothelial adhesion on a fibrinogen surface. **Objectives:** Study the potential of GST-INS to inhibit the adhesion of HUVECs (Human Umbilical Vein Endothelial Cell- that overexpressed αvβ3) to platelets and the ability of this molecule to induce HUVECs detachment. **Methods**: Plates were coated with platelets for 2 h and washed to remove non-adherent platelets. HUVECs were then pre-incubated for 30 min with GST-INS, GST or Agrastat (Ag) (a commercially selective inhibitor of αIIbβ3 integrin) and added to platelets for 40 min. After washing, adherent cells were analyzed by microscopy. For detachment assay, HUVECs or fibroblastic cells (L929) were seeded in culture plates. After 24hs, cells were incubated with 1 μM GST-INS, GST, Ag or culture medium. After 24hs, the cells were washed and the adherent cells were stained and analyzed by microscopy. **Results and Discussion**: GST-INS inhibited HUVECs adhesion to platelets at concentration as low as 0.32 μM. This inhibition was dose dependent and significantly higher than the inhibition induced by Ag or GST. In the detachment assay, the treatment of HUVECs with GST-INS led the majority of the cells to detachment, while the remaining showed retracted morphology. HUVECs were not detached by GST or Ag treatment. Differently, GST-INS, GST and Ag did not induce detachment of L929, used as control. In conclusion, our results suggest that GST-INS showed bifuncionality and selectivity towards αIIbβ3 (platelets) and αvβ3 (HUVECs) integrins, since it inhibited platelet/endothelial cells adhesion and induced HUVECs detachment. These results may be an important approach to inhibiting tumor growth, angiogenesis, and metastasis.

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12.38 Antimicrobial activity from ticks eggs waxes

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Introduction: Ticks lay their eggs in the environment, and involve the eggs in a waxy layer to protect them from desiccation and microbial attack. This wax is produced by an organ known as Gene´´s Organ. Bioprospection has shown the presence of active molecules in the hemolymph of arthropods as well as in the salivary glands of ticks. Some of these molecules are of interest for the development of new pharmacological drugs. In this study, different tick species from colonies maintained in our laboratory were used to test the antimicrobial effect of the organic extract present in the composition of the wax envolving the eggs. **Objectives**: The objective of this study is to evaluate the antimicrobial effect of the organic extract from eggs of the following tick species: *Amblyomma cajennense*, *Amblyomma aureolatum*, *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus sanguineus*. **Methods:** The wax was extracted by using chloroform/methanol (2:1), 1.0 mL for each gram of egg masses. We tested the organic extract from *A. cajennense*, *A. aureolatum*, *R.* (*Boophilus*) *microplus* and *R. sanguineus*. The antimicrobial activity was evaluated by the standard method of disc diffusion stablished by Kirby and Bauer (Bauer et al., 1966). The microorganisms used to test the activity were: *C. albicans, M. luteus, E. coli* and *S. aureus*. After incubation, plates were observed for the presence or absence of growth inhibition. **Results and Discussion:** The organic extract of the *A. aureolatum* and *R. sanguineus* showed an inhibition zone for the strains of *C. albicans*, *M.luteus*, *E.coli* and *S.aureus*. This result is in accordance with the antimicrobial activity reported for the wax extracted from other ticks.

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12.39 Purification of polysaccharide type 1 from *Streptococcus pneumoniae* in the presence of detergent sodium dodecyl sulphate

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Introduction:*Streptococcus pneumoniae* is a pathogenic bacterium that causes pneumonia, meningitis, acute otitis, sepsis and bacteraemia. The major pneumococcal virulence factor is the capsular polysaccharide (PS) that permits to classify this bacterium in 93 serotypes. The pure PS are components of the vaccines, which are the best strategy to prevent infections. However, PS do not produce cross protection, for this reason, commercial vaccines have up to 23 different PS. In Brazil, the serotype 1 is the third most important cause of infections and PS type 1 (PS1) is an obligatory antigen in pneumococcal vaccines. The purification of PS1 consists of cross flow ultrafiltration, fractional ethanol precipitation and hydrolytic enzyme treatment. The purity required demanded by the World Health Organization (WHO) for PS1 is that it should not contain more than 3 % (w/w) of protein (Prt) and not more than 2 % (w/w) of nucleic acids (NA). **Objectives:** Evaluate the purification process of PS1 in the presence of sodium dodecyl sulfate (SDS) in order to obtain PS1 with the purity required, maximizing recovery and minimizing costs. **Methods:** Microfiltrated fractions from different cultures, previously concentrated by tangential ultrafiltration in 50 kDa spiral membrane, were combined in a single Pool fraction, which was divided in aliquots and stored at -25 °C. A portion was divided into two, with and without 0.5 % SDS, and precipitated with 20 % ethanol. After centrifugation, the supernatants containing PS1 were re-precipitated with 45 % ethanol. The insoluble PS1 was recovered by centrifugation and dissolved with distilled water. The water-soluble fractions were treated with hydrolytic enzymes (nuclease and proteases). The hydrolyzed material was removed by tangential ultrafiltration in 50 kDa cassette membrane. Prt was quantified by Lowry, NA by absorbance at 260 nm and PS1 by sandwich ELISA. **Results and Discussion:** In the purification process without SDS, PS1 was obtained with final yield of 23 % in relation to the initial Pool fraction, residual contamination of 19 % Prt and 12 % NA. Sample processed with SDS was obtained with a global yield of 33 % PS1 with 21 % of residual Prt and 11 % of NA. The results show that purity requirements of WHO were not reached. Nevertheless, it can be seen that sample with SDS had better recovery of PS1 (33 % vs. 23 %). Further experiments are been conducted to test different conditions with SDS in order to improve the purification process and reach the required purity.

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12.40 Presence and distribution of antibodies against Shiga toxins in bovine sera

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are enteric pathogens that can cause bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans. The STEC major virulence factors are two potent phage-encoded toxins called Stx1 and Stx2, a family of structurally-related cytotoxins with similar biological activity. Cattle are considered a primary reservoir of these organisms, followed by sheep, goats and wild ruminants. Fecal shedding from cattle is an important source of contamination and transmission. Outbreaks occur through ingestion of contaminated food or water. In Brazil, these organisms were present in 10% to 83% of the herd, depending on the region studied and there is no information about the presence of antibodies against Shiga toxins in the bovine sera. **Objectives:** Investigate the presence of antibodies against Stx1 and Stx2 in a collection of sera from cattle of different Brazilian farms. **Methods:** Specific antibodies to Stx1 and Stx2 were determined by indirect ELISA. Microplates were coated with Stx1 or Stx2 at 1 µg/mL in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6, at 4 ºC for 18 h and blocked with 1% BSA at 37 °C for 30 min. Then, 100 µL of twofold serial dilutions from each serum sample in duplicate, starting from 1:16, were added and incubated at 37 °C for 2 h, followed by incubation with peroxidase-conjugated rabbit anti-bovine IgG diluted 1:5,000, at 37 °C for 1h. The reactions were developed with 0.5 mg/mL OPD plus H_2O_2, and stopped by the addition of 1 N HCl. The absorbance was measured at 492 nm in an ELISA reader. The last serum dilution yielding an absorbance value of 0.1 over background was recorded as the end point titer for each sample. The reactivity of antibodies to purified Stx1 and Stx2 toxins was also tested by immunoblotting using selected sera. Briefly, 10 µg per slot of toxins were submitted to 15% SDS-PAGE. After electrophoresis, the proteins were transferred to a nitrocellulose membrane. The membrane was blocked and incubated with each of the selected sera. Next, the membrane was washed and incubated with peroxidase-conjugated rabbit anti-bovine IgG. After washing, DAB plus H_2O_2 were added and the reaction was stopped after 15 min by the addition of distilled water. **Results and Discussion:** All 59 sera tested by ELISA presented antibodies against Stx1 and Stx2 and had titers ranging from 2048 to 16384. Among the sera, titers were similar for both toxins in 30 samples, higher against Stx1 in 14 and lower for Stx1 in 15 samples. The selected sera tested by immunoblotting reacted with the A subunit of the toxins. Additional investigations are needed to verify if these antibodies can neutralize the toxins and consequently protect against Stx.

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12.41 Anti-inflammatory effects of Mygalin (Acylpolyamine) in LPS-stimulated macrophages

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Introduction: Natural polyamines (putrescine, spermine and spermidine) are essential elements of cells from all species. They have low molecular weight and are implicated in many cellular processes, including DNA replication, apoptosis, transcription and translocation and modulation of intracellular signs. The biosynthetic pathway of polyamines has been target of research in attempt to control tumor cells and pathogenic agents. Recently, an acylpolyamine was isolated from the hemocytes of the spider *Acanthoscurria gomesiana*, and identified as a spermidine analogous, named Mygalin. The macrophages activation with Mygalin increases NO and TNF-α but not IL-1β. We evaluated the effects of synthetic Mygalin associated or not with additional signaling molecules as an immunomodulator of the innate immune response. **Objectives:** Investigate *in vitro* the effects of Mygalin in LPS-stimulated macrophages and the involvement of TLRs. **Methods:** Bone marrowmacrophages obtained from femur and tibia of C57BL/6 mice and TLR4 $^{-/-}$differentiated for 7 days were pre-stimulated with Mygalin (5-40 ug/ml) 1 hour before the addition or not of LPS (1 ug/ml) and zymozan (1000 ug/ml). After 20 hours, supernatant was collected for cytokine and chemokine analyses (IL-6, IL-10, MCP-1) by ELISA and nitrite level by Griess reaction. **Results and Discussion:** The production of TNF-α was increased, however no significant differences was seen in the IL-12p40, IL-6 and MCP-1 synthesis. The association of Mygalin with Polymixine B did not change the TNF-α synthesis.Mygalin inhibited significantly the production of the pro-inflammatory cytokine IL-6 and the chemokine MCP-1 induced by LPS, as it did in previous results with TNF- α and IL-12p40. It also inhibited the production of nitric oxide in C57BL/6 and TLR4 $^{-/-}$ mice induced with zymozan, indicating the possible involvement of TLR2 in signaling pathways. Our results suggest that Mygalin should be further explored asanimmunomodulator of macrophages activity and can be investigated as a strategy in the control of infections.

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13 PIBIC-EM

13.01 On the reproduction of *Sibynomorphus mikanii*

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Introduction: Snakes, like lizards, have two basic types of reproduction, depending on the species: either they are oviparous, and lay their eggs in places protected from the sunlight, or viviparous, with the youngsters developing inside the female's body. In the oviparous species development of the embryos occurs outside the mother's body, inside eggs and deriving its nutrition from the yolk sac. The egg shell may be flexible as in snakes, or rigid like in most lizards, furnishing mechanical protection against impacts, and allowing gas exchange (exit of the carbonic gas and entrance of oxygen) with the environment. The snake *Sibynomorphusmikanii* is an oviparous species commonly known as "dormideira". It is a terrestrial snake with nocturnal habits and feeds mostly on slugs. It is very common from Pará State to Rio grande do Sul. **Objective:** The objective of this study was to analyze some aspects of the external morphology of the eggs from a specimen of *S. mikanii* kept in captivity at the Museu Biológico of the Instituto Butantan. **Methods:** The eggs were all part of the same litter (a total of 6 eggs). After the female laid the eggs, those were collected, numbered and measured, and the individual weight was recorded. They were kept in polypropylene boxes measuring (15 × 11 × 11 cm) lined with vermiculite, and kept in an egg incubator with controlled humidity and temperature. **Results and Discussion:**The eggs of *S. mikanii* are elongated, whitish and with a flexible shell. Their mean weight was 1,8g, and the mean lenght and width were respectively 24 mm and 12.8. Oviposition season (November) and number of eggs (6) observed in captivity correspond to the described in the literature for the species (breeding between September and February, and litter size from 1 to 10 eggs). Studies on the reproductive season and morphological description of the snake's eggs are fundamental for the knowledge about the reproductive patterns of each species.

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13.02 Relationship between interfang distance and snake length

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Introduction: Snake size and patient mass are very important factors influencing snakebite severity and treatment protocols.The patient's description of snake size can be helpful but the distance between fang marks can determine more precisely the size of the offending snake. As *Bothrops jararaca* and *Crotalus durissus* are the pitvipers frequently involved in human and animal snake bite in Brazil, they were the species chosen for this study. **Objectives:** Determine the relationship between interfang distance and the snout-vent length (SVL) of these species of snakes. **Methods:** Seventeen specimens of *B. jararaca* (males and females) and 39 specimens of *C. durissus* (males and females)recently-caught from nature were used in this study. One week after their arrival at the Laboratório de Herpetologia, the snakes were submitted to venom milking with the aid of carbon dioxide. The extraction marksleft on a plastic-covered beaker were measured with digital calipers. For each specimen, we also measured SVL (to the nearest mm). At the moment our sample only included juveniles and fully mature snakes of each species, but neonates will also be studied afterwards. **Results and Discussion:** We verified that the interfang distance was positively correlated with snake's SVL in both species. In *B. jararaca* the correlation coefficient was 0.58; $r^2 = 0.33$, $p < 0.01$ and in *C. durissus* the correlation coefficient was 0.79; $r^2 = 0.62$, $p < 0.0001$. These results show the possibility of developing a chart where, after measuring the wound puncture in a human or animal patient, the doctor or veterinarian would be able to know the approximate size of the snake and, consequently, the severity of the ophidian accident.

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13.03 Bioactive Molecules in Diplopods (*Rhinocricus* sp.)

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Introduction: Diplopods date from the pre-Cambrian period. They are found in all regions except Antarctica. This happens because the arthropods are well adapted to most environments, even the hostile ones with high presence of microorganisms and parasites, what may indicate an efficient immune system. Bioactive molecules are an important factor in the innate immune system. These molecules can be constitutive or synthesized after an infection. The study of bioactive molecules has been gaining popularity as they may be the best substitutes for antibiotics, and may also provide necessary data for a better understanding of the immune system of these animals. These molecules have been purified and characterized in a broad range of invertebrates, but the Diplopoda group calls attention since is poorly studied. **Objectives:** Identify antimicrobial molecules from diplopods. **Methods:** The animals were collected, and separated into experimental and control groups. The experimental group was challenged withbacterial solution, and the control group was inoculated with saline solution. The hemolymph and full body extract of the both groups were pre-purified with Sep-Pak cartridges and after the elutions (5, 40, 80% ACN) were purified by RP-HPLC. The fractions were tested against yeasts, Gram-negative and Gram-positive bacterias by Growth Inhibition in Liquid Broth tests, in order to evaluate antimicrobial activity. The fractions that showed bioactivity passed by mass analyses and "de Novo" sequencing. **Results and Discussion:** In the plasma of the not challenged Diplopods I've discovered a new bioactive molecule, a peptide. This molecule, *Rhinocricrin*,has a low molecular weight, likely 2368.69 Da; it inhibits the growth of Gram negative bacteria *E. coli* (SBS363) and does not cause hemolysis of human erythrocytes, which may indicate also that is not cytotoxic, making it promising as an active principle in the development of a new antibiotic drug. Also it resembles fragments of hemocianins of the super family C of spiders. Such similarity suggests that, within the phylum Arthropoda, the subphylum Miriapoda lies closer to the subphylum Chelicerata than to subphyla Crustacea and Hexapoda. In the full body extract I've discovered a new non-peptide molecule that is active against Gram-negative bacteria *E. coli* (SBS363), *E. cloacae* and *S. serovar*. With its highest absorbance at the wavelength 290nm. and a probable mass of 500Da, this molecule dubbed *Diplopodin.* It is promising as an active principle in the development of a new antibiotic drug, and may be the key to understanding the immune system of these animals, since it corroborates the hypothesis that their bioactive molecules are also synthesized after infection.

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13.04 Capsular polysaccharide produced by *Haemophilus influenzae* type B: a protector molecule

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Introduction.*Haemophilus influenzae* type b(Hib) is a Gram-negative, cocobacillar bacterium. Generally, it is aerobic, but can growth as a facultative. The most prevalent group worldwide is the serotype b, causing bacteremia, pneumoniae and bacterial meningitis in infants, young children and elderly.*H. influenzae* is an opportunistic pathogen and the invasion may occur in consequence of viral infections, reduced immune function or chronicall infection in the tissues. Capsular polysaccharide (PRP) produced by this microorganism is the most external molecule and constitutes the main virulence factor,offering protection against macrophages from the host. On the other hand, capsular polysaccharide is used as antigen in the Hib vaccine. **Objectives**: To understand how the capsular polysaccharide can protect the bacterium in the course of infection and the human being in the vaccine formulation. **Methods:***Haemophius influenzae* type b strain GB3291 was cultivated in a reactor of 10 liters. The culture broth centrifuged at 17.000g during 1 hour at 4°C to remove bacterial cells and the polysaccharide was isolated from the supernatant. The sample containing polysaccharide was submitted to the following tratments: 1) addition of ethanol in different concentrations; 2) addition of a cleaning agent (detergent) in combination to ethanol. Insoluble impurities were removed by centrifugation at 12.000 g during 15 min. A second ethanol fractionation was performed to isolate the PRP. As the cleaning agent, three detergents were tested: a) sodium deoxycholate; b) sodium dodecyl sulfate; c) sodium taurodeoxycholate. In each experiment, polysaccharide was measured by Bial´s method. **Results and Discussion:** In the culture broth there are many components such as proteins, nucleic acids, lipids and glycoproteins from the medium itself and from metabolites generated by Hib, some cellular debris and integer cells. In order to remove the impurities, a physico-chemical properties from these molecules is taken into consideration, such as solubilities and hydrofobicities. Ethanol at 30% removes the majority of nucleic acids and proteins, and detergents help to remove proteins, by denaturating them, and LPS. The most effective cleaning agent was SDS, a strong detergent, which removed more than 80% of the contaminants. purifying the PRP. This purified PRP is the main component used in the Hib vaccine.

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13.05A journey in the *Haemophilus influenzae*`s world

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Introduction:*Haemophilus influenzae* (Hi) known as Pfeiffer`s bacillus, is a Gram-negative and pleomorphic bacterium. Generally is aerobic, but can growth as a facultative. There are two major categories of *H. influenzae*: the unencapsulated (nontypeable) and the encapsulated strains (typeable). Encapsulated strains were classified based on their distinct polysaccharide antigens. Six serotypes were recognized as encapsulated *H. influenzae*: a, b, c, d, e, and f, in which serotype b is the most prevalent. The majority of *H. influenzae* is opportunistic pathogens and usually lives in their host without causing disease. However, when some episode in the host takes place, such as viral infection, reduced immune function or chronically infected tissues, an opportunity of invasion is created. Naturally acquired disease caused by *H. influenzae* occurs in infants and young children causing bacteremia, pneumonia and acute bacterial meningitis. **Objectives**: To make a small journey in the *Haemophilus influenzae*'s world to know its microbiology, morphology, virulence and some growth factors. **Methods:***Haemophius influenzae* type b strain GB3291 was used in the following experiments: 1) Gram stain and microscopic observation; 2) Cultivation of Hib in chocolate agar plates, as a control, and BHI agar plate in the presence of NAD (nicotinamide adenine dinucleotide), Hemin and a combination of both to evaluate the bacterial growth (plates were incubated at 37°C in a chamber jar with CO_2 of 5-6% during 24 hours). 3) Characterization of Hib was carried out by using two culture media (a complex MP medium and a chemical defined one); the growth was followed every hour and one sample was collected in the last hour for polysaccharide measurement. This culture was used also for the biochemical serial test using API-20e and API NH kit. 4) Colorimetry based on Bial´s method was used for polysaccharide determination. **Results and Discussion:** In the microscopy analysis, Hib presented rod cocco bacillar shape and in some cases filamentous, showing its pleomorphism. The growth was visible in the chocolate agar plate, which contains NAD, Hemin and horse blood; on the other hand, in the BHI agar plate the bacterial cell was developed indispensablyin the sample containing both Hemin and NAD. The complex medium presented $DO_{540\ nm}$ of 7.18 higher than defined one with value of 3.5; and polysaccharide concentration of 330 mg/L and 210 mg/L for complex and defined medium respectively.

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13.06Antimicrobial peptides in the venom of *Tityus serrulatus*

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Introduction: Scorpions certainly form one of the most remote groups of arachnids to conquer the Earth's surface. Scorpion venoms are complex mixtures in which toxic polypeptides constitute the most prominent components, known fortheir potencythe venomthat is composed ofseveral proteins,includingneurotoxins, present evidence supporting the ubiquitous presence of polypeptides and their precursors in scorpions of the genus *Tityus*. In recent years, many antimicrobial peptides have been found in the venom of scorpions. Antimicrobial peptides (AMPs) are small, cationic molecules that form the innate defense mechanism of many organisms. The AMPs appear as an ideal alternative to a fast and efficient defense against microbes. **Objective:** The objective of thisstudy was to identify new antimicrobial peptides in the venom of the scorpion *Tityus serrulatus*. **Methods:** Thevenom was obtained from 56 animals by electrical stimulation, and then dissolved with water, centrifuged, and the soluble part was dried by vacuum centrifugation and reconstituted in 2 ml of acidified water (TFA 0.05 %). The soluble part was applied in HPLC reverse-phase chromatography on a semi preparative Jupiter C18. Elution was performed using a linear gradient 0-80% ACN/TFA 0.05% in 60 min at a flow rate of 1.5mL/min. The antimicrobial activities were determined by liquid growth inhibition assays against Gram-negative bacteria *Escherichia coli* SBS363,Gram-positive bacteria *Micrococcus luteus* A270and yeast *Candida albicans* MDM8 and the minimal inhibitory concentration was determined too. The fractions that presented antimicrobial activity were submitted to SDS-PAGE and mass spectrometry.**Results and Discussion:** Our results showed nine fractions with antimicrobial activity against all microorganisms tested. These fractions were submitted to SDS-PAGE to determine homogeneity and each fraction was cut in little slices, treated (reduced, alkylated and trypsinizated), submitted to mass spectrometry and compared with databases. At this moment, we found one molecule with antimicrobial activity against *M. luteus* and *C albicans* and identity with a neurotoxin TS8 and this fraction has a fragment sequenced. The purification and characterizationof these peptides are still in progress. These results show us that these animals have mechanism to paralyze the prey and the same time to avoid the infections by ingests the prey how the first line to defense them.

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13.07 Seasonal fecal exams inrecently-caught *Bothrops jararaca* and *Crotalus durissus*snakes (Ophidia, Viperidae)

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Introduction: Free-living snakes host a wide variety of protozoan and metazoan endoparasites, although there are no epidemic reports associated with parasitism in wild populations. When the animals are deprived of liberty, one of the most stressful conditions that a wild animal may be exposed, it is expected that parasites taken as commensals, may become opportunistic pathogens, difficulting maintenance in captivity. The consequences of parasitism are competition with the host for food, tissue reaction, obstruction of blood and lymphatics vessels, edema, ulceration, necrosis and anemia, that may lead the animal to death. **Objectives**: Evaluatethe seasonality of protozoans and metazoans in the stool of recently-caught pitvipers and rattlesnakes, using qualitative and quantitative methods. **Methods**: Stool samples were collected through cranio-caudal massage of the snake and stored at 4°C until being processed. Qualitative methods used were Willis Technique and Water-EtherTechnique, and the quantitative method used was the McMaster technique. In Willis Technique, feces are diluted in saturated sugar solution and rest for 20 minutes in a recipient with a cover slip. After twenty minutes the coverslip is placed on a slide and examined under a light microscope. In Water-EtherTechnique, 1 mL of feces is diluted in distilled water and mixed with the same amount of ether. The solution is centrifuged, the supernatant discarded and the precipitate examined by light microscopy. In both techniques, the eggs are identified as trematodes, cestodes or nematodes. Oocysts of protozoa are also identified. In the McMaster Technique, an accurate amount of feces is diluted in saturated sugar solution and placed in the two compartmentsof the McMaster chamber.All the eggs are counted in both compartments, divided by two and the result multiplied by 100, giving the number of eggs/g of feces. **Results and Discussion**: As the study began only in April, we still do not have the parasites seasonality results, but we observed that nematode eggs were the most commonly found parasite in the fall and winter seasons for pitvipers and rattlesnakes, followed by the protozoan oocyst,*Caryospora* sp.

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